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CEREAL CHEMISTRY

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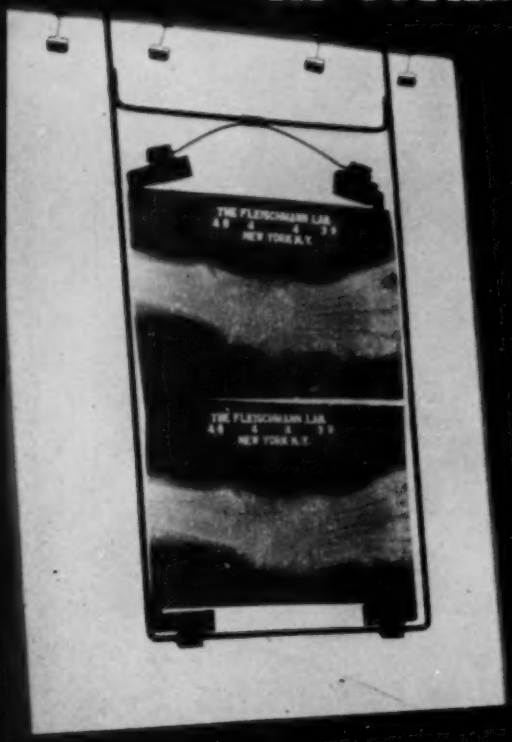

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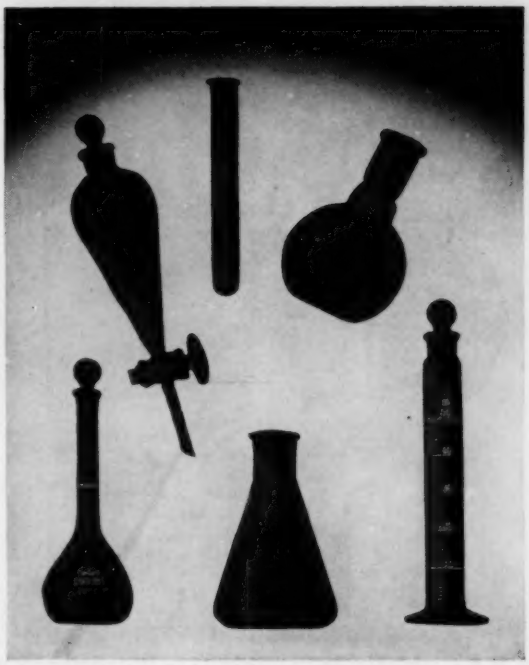

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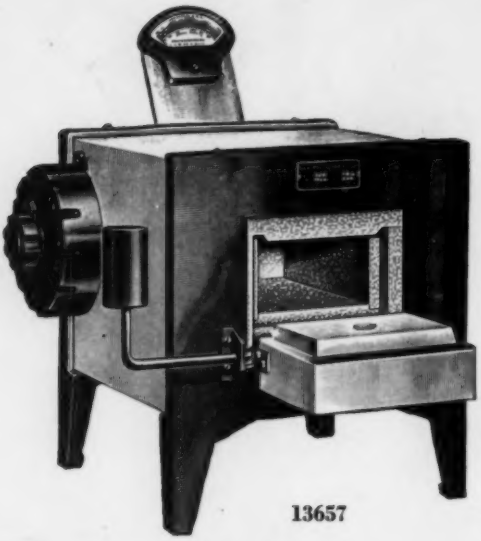

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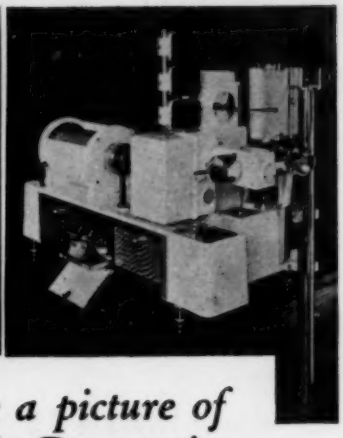

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CEREAL CHEMISTRY

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THE THIAMIN, RIBOFLAVIN, NICOTINIC ACID, AND PANTOTHENIC ACID CONTENTS OF WILD RICE (ZIZANIA AQUATICA) [1]

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(Received for publication January 29, 1942)

Kennedy (1924) found that wild rice is an adequate source of B vitamins for normal growth of rats at 86% level in the diet but no analyses have been reported of the individual components of the vitamin B complex in this cereal since the discoveries regarding their chemical identity and the perfecting of simple, accurate methods for their quantitative determination. The present study was undertaken at the request of Mr. James W. Kauffman of the U. S. Department of the Interior, Office of Indian Affairs Field Service, Minneapolis, Minnesota, because of the importance of wild rice as a native crop harvested by the Indians of Minnesota. The samples analyzed were obtained by Mr. Kauffman and are representative of the parched product processed by the Indians and marketed by them.

Thiamin was determined by the thiochrome method of Hennessy (1941), riboflavin by the microbiological method of Snell and Strong (1939) as modified by Andrews, Boyd, and Terry (1941), nicotinic acid by the microbiological method of Snell and Wright (1941), and pantothenic acid by the microbiological method of Strong, Feeney, and Earle (1941).

The results shown in Table I indicate that parched wild rice may be regarded as a good source of thiamin, riboflavin, and nicotinic acid for human nutrition. It appears to be a surprisingly good source of nicotinic acid since 100 g contain about 40% of the recommended 15 mg daily allowance for a person requiring 2500 kilo-calories.[2] The wild rice compares favorably with wheat, corn, and rye in thiamin content and is considerably richer in riboflavin than wheat, corn, oats, or rye.

[1] Paper No. 1961, Scientific Journal Series, Minnesota Agricultural Experiment Station.
[2] Recommendations of the Committee on Food and Nutrition, National Research Council, National Nutrition Conference, Washington, D. C., May 26–28, 1941.

TABLE I

B-COMPLEX VITAMINS IN WILD RICE

Sample	Crop year	Vitamin content per 100 g			
		Thiamin	Riboflavin	Nicotinic acid	Pantothenic acid
		mg	*mg*	*mg*	*mg*
No. 1	1939	0.507	0.542	6.69	0.98
No. 2	1939	0.579	0.700	5.48	1.31
Screenings	1939	0.371	1.058	6.34	1.41
Nett Lake	1940	0.293	0.417	6.58	0.65
Nett Lake (light)	1940	0.371	0.708	5.58	1.67
Mitchell Dam	1940	0.600	0.800	6.44	0.42
Average (exclusive of screenings)	—	0.470	0.633	6.13	1.01

Literature Cited

Andrews, J. S., Boyd, H. M., and Terry, D. E.
1941 Application of the microbiological method to the riboflavin analysis of cereals. Abstracts Division of Biological Chemistry, Am. Chem. Soc. 102nd Meeting, Atlantic City, Sept. 8–12, p. 36.

Hennessy, D. J.
1941 Chemical methods for determination of vitamin B_1. Ind. Eng. Chem. (Anal. Ed.) **13**: 216–218.

Kennedy, Cornelia
1924 The nutritive properties of wild rice (*Zizania Aquatica*). J. Agr. Res. **27**: 219–224.

Snell, E. E., and Strong, F. M.
1939 A microbiological assay for riboflavin. Ind. Eng. Chem. (Anal. Ed.) **11**: 346–350.

Snell, E. E., and Wright, L. D.
1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem. **139**: 675–686.

Strong, F. M., Feeney, R. E., and Earle, Ann
1941 Microbiological assay for pantothenic acid. Ind. Eng. Chem. (Anal. Ed.) **13**: 566–570.

A CALCULATED BLANK FOR THE ESTIMATION OF THIAMIN BY THE FERMENTATION METHOD

I. ENRICHED BREAD

GEORGE J. LAEMMLE and O. W. BARLOW

Research Laboratories, Winthrop Chemical Company, Inc., Rensselaer, New York

(Received for publication May 23, 1942)

In the assay of thiamin by the yeast fermentation procedure developed by Schultz, Atkin, and Frey (1937), the apparatus described consists essentially of twelve flasks shaken in a constant-temperature bath and connected to twelve gasometers. Since known amounts of thiamin are placed in two of the flasks, and since the assay of each

sample requires the simultaneous determination of the sulfite blank of the sample in the same apparatus, as shown by Schultz, Atkin, Frey, and Williams (1941), no more than five samples can be assayed at one time. We have found it possible to complete two separate fermentation runs in an eight-hour day, permitting the assay of as many as ten samples for thiamin content by this procedure as a matter of daily routine. In this respect the yeast fermentation method suffers in comparison to thiamin assay by the thiochrome procedure described by Hennessy and Cerecedo (1939) and modified by Hennessy (1941) and by Connor and Straub (1941). Sixteen to twenty samples a day may be assayed by this method.

The omission or elimination of the sulfite blank in the yeast fermentation method would not only double the capacity of the apparatus employed, but would also result in a considerable decrease in the time required for a fermentation run, since much of the assay time represents the preparation of the sulfite blank. This blank, however, cannot be disregarded as it often comprises a very considerable part of the total fermentation-stimulating substances. Nor is the blank constant for any class of material assayed but varies with different samples and even at times with duplicate determinations, although the *net* thiamin in each of the duplicates is in close agreement. We have observed this variability of the sulfite blank in all kinds of material assayed by this method.

In laboratories where the same material, prepared according to an unchanging process and formula, is routinely assayed, it may be feasible to omit the sulfite blank and to substitute a standard sample of the product which has been carefully assayed by one or more methods. All the samples are then corrected by the factor necessary to adjust the apparent thiamin content of the standard to the known true value. In laboratories where many different materials are assayed, or where the same general material is received from different sources, such a procedure cannot be adopted.

We have for some time observed a limited relationship between the sulfite blank and the total apparent thiamin (or total fermentation-stimulating substances). When two different samples of the same general material are assayed by the fermentation procedure, the sample having the greater apparent thiamin content will usually have the greater sulfite blank. This is especially noticeable with a material like enriched bread, wherein the variation in thiamin content among individual samples is greater than that encountered in pharmaceuticals produced under controlled conditions, or in unfortified natural materials. When, therefore, it seemed possible that this ratio between the sulfite blank and the total apparent thiamin, if compiled for a

large number of samples of the same general material, might prove to be constant within the experimental error of the method itself, enriched bread appeared to be the material best suited to investigation.

Experimental

The ratio between the sulfite blank and the total apparent thiamin, both figures being expressed in micrograms of thiamin per gram of air-dry sample, was determined for 100 unselected samples of enriched bread. In order to obtain the most trustworthy picture of variations in this ratio, the series was made up of breads taken in the order received for assay without omission of any sample. The frequency distribution of the ratio blank: fermentation-stimulating substance (f.s.s.) for this series of breads is shown in Figure 1.

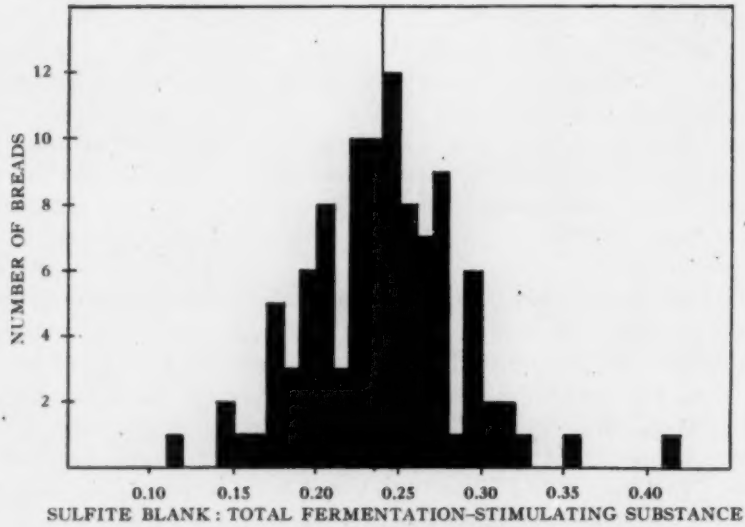

Fig. 1. Frequency distribution of the ratio of sulfite blank to total f.s.s. Average ratio, 0.238. $\sigma = 0.047$.

The frequency distribution of this ratio did not afford evidence of any constancy sufficient to permit the use of an empirical correction factor, determined in such fashion, in place of the sulfite blank. The standard deviation of the series was almost 20% of the mean value, and the individual ratios ranged from a low of 0.109 to a high of 0.411.

There were, however, two encouraging aspects of the frequency distribution. First, the distribution followed fairly closely that predicted from the normal curve of error, as shown in Table I. This agreement is evidence that the variation in the ratio of the blank to the total fermentation stimulation is due to chance unavoidable errors

TABLE I

VALUES FOR NORMAL ERROR CURVE

Deviation from the mean in terms of σ	Number of breads in range of error predicted from normal curve	Found
$\pm 0.6745\,\sigma$	50	54
$\pm \sigma$	68	72
$\pm 1.4\,\sigma$	84	87
$\pm 1.7\,\sigma$	91	93
$\pm 2.2\,\sigma$	97	97

in the method and technique, rather than to real differences in the ratio among individuals in the series. Second, and more important, is the fact that the blank averages only 24% of the total increase in the fermentation rate. Therefore a comparison of the thiamin content of the members of the series determined with the factor 0.238 f.s.s. as the blank, with the thiamin content as determined in the usual manner, will result in a much smaller standard deviation than that obtained when the same series was used to determine the average ratio, 0.238. This fact is demonstrated graphically in Figure 2.

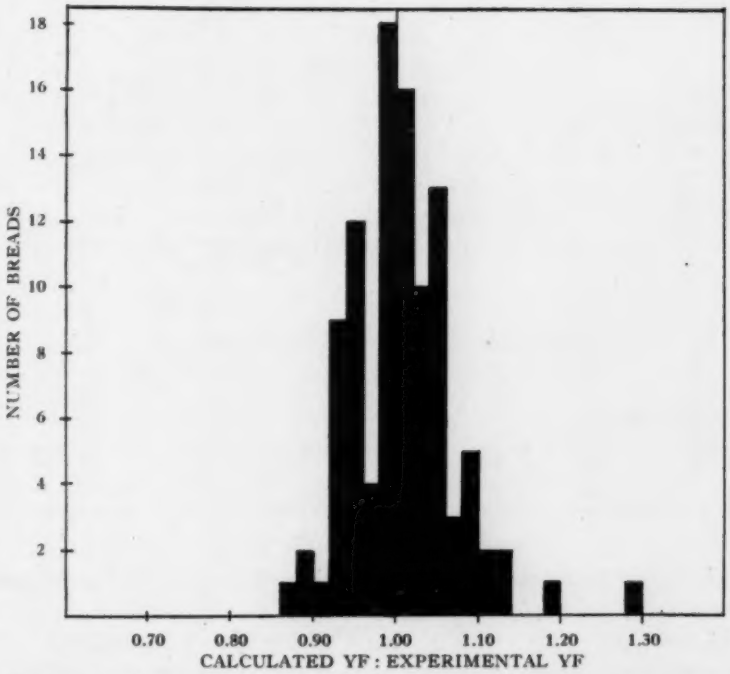

Fig. 2. Frequency distribution of the ratio of thiamin contents determined from the calculated blank (0.238 f.s.s.) and from the actual sulfite blanks. Average ratio, 1.002. $\sigma = 0.063$.

In this comparison, the calculated and experimental methods agreed within ±5% for 70% of the series, and they agreed within ±10% for 92% of the series.

It is important to realize that in this effort to establish a sound basis for the use of a calculated blank, by statistical methods, we are not studying the distribution of experimental observations around a known true value. Rather, we have a picture of the variance of a series of calculated results about an experimentally determined mean which is subject to all the uncertainties inherent in the method and in the technique of the analyst. Therefore it is possible that the calculated blank, based on a large number of determinations, is more reliable than the individual experimental blank which it supplants. This possibility was studied in the following way.

Ten enriched breads which were not members of the series previously studied were assayed by the yeast fermentation method, employing the usual procedure for determination of the sulfite blank. The same group of breads was then assayed by the same procedure, omitting the sulfite blank. The blank in this case was calculated as 0.238 f.s.s. Finally the ten breads were assayed by the thiochrome method. The results obtained by the three procedures are tabulated in Table II.

TABLE II

THIAMIN ASSAY BY THREE METHODS

Sample No.	Thiamin air-dry basis			Thiochrome / Sulf YF	Deviation	Thiochrome / Calc YF	Deviation	Calc YF / Sulf YF	Deviation
	Sulf YF	Thiochrome	Calc YF						
	μg/g	μg/g	μg/g	%	%	%	%	%	%
1608	3.16	3.03	2.90	96	− 4	104	+ 4	92	−8
1611	3.18	2.96	3.18	93	− 7	93	− 7	100	0
2098	2.98	2.88	2.85	97	− 3	101	+ 1	96	−4
2099	3.23	3.38	3.29	104	+ 4	103	+ 3	102	+2
2188	4.69	4.49	4.59	96	− 4	98	− 2	98	−2
2193	3.55	3.69	3.55	104	+ 4	104	+ 4	100	0
2253	2.71	2.60	2.76	96	− 4	94	− 6	102	+2
2254	2.88	2.60	2.90	90	−10	90	−10	99	−1
2289	3.69	3.46	3.49	94	− 6	99	− 1	94	−6
2335	3.19	3.15	3.18	99	− 1	99	− 1	100	0
Average	3.33	3.22	3.27	96.9	± 4.7	98.5	± 3.9	98.3	±2.5

Sulf YF = yeast fermentation with sulfite blank. Calc YF = yeast fermentation with calculated blank.

The closest agreement found was that between the fermentation assay with the calculated blank and the usual fermentation procedure. The former procedure yielded results which were in somewhat closer agreement with those obtained by the thiochrome method than were the latter, but the deviation in all three assays is sufficiently within

the experimental error of the methods to preclude the assumption of a significant difference in the reliability of any of the procedures. However, the calculated blank stands up well in comparison with another standard assay method.

In a preliminary study of a limited number of non-enriched breads we have found the ratio between the sulfite blank and the total fermentation-stimulating substances to be considerably higher than that found for the enriched breads studied. Such a finding suggested that this ratio would decrease as the enrichment level of the bread was increased. However, a breakdown of the series from which the average ratio was calculated, according to thiamin content, demonstrated no essential variation in the ratio over a considerable range of enrichment (Table III).

TABLE III

EFFECT OF ENRICHMENT LEVEL

Thiamin	No. breads	Calc YF / Exptl YF	Average deviation
μg/g		%	+
1–2	2	104.5	± 0.5
2–3	39	102.1	± 5.6
3–4	54	98.4	± 3.9
4–5	1	99	− 1
8–9	2	103	± 1
12–13	2	92.5	± 0.5

A comparison of the ratio calc YF : exptl YF obtained with those members of the series known to have been fortified with enriched yeast and with those breads known to have been enriched with crystalline thiamin, revealed no significant difference in either the mean ratio or average deviation of the mean (Table IV).

TABLE IV

EFFECT OF METHOD OF ENRICHMENT

Number of breads	Method of enrichment	Calc YF / Exptl YF	Average deviation
13	Crystalline thiamin	0.976	± 0.060
15	Enriched yeast	1.032	± 0.055

It is unfortunate that for the majority of the series the method of enrichment was unknown or not known with certitude.

Conclusions

It is to be expected that the empirical ratio determined from the series of enriched breads will change in value with the inclusion of a

greater number of breads in the series, or when calculated from a new series of experimental results. However, the agreement in results obtained with the experimental and the calculated sulfite blanks, illustrated in Table IV, makes improbable any change in the factor considerable enough to invalidate its use. It is also possible that this ratio may differ widely among laboratories while remaining fairly constant for each. This variation, if demonstrated, will severely limit the practical value of the calculated blank, but we believe that the agreement between laboratories in collaborative studies renders improbable such a variation. We are hopeful that a constant empirical ratio between the blank and the total apparent thiamin in the yeast fermentation method can be established, not only for enriched bread but for other assay materials, with a resulting increase in the capacity of the method and decrease in the assay time involved.

Summary

The ratio of the sulfite blank to the total fermentation-stimulating substances, in a series of 100 enriched bread samples assayed for thiamin by the yeast fermentation procedure, was found to be 0.238. Substitution of this empirical factor for the individual sulfite blanks doubles the capacity of the method and brings about a considerable reduction in assay time. The results obtained with the calculated blank are in close agreement with those obtained in the usual way.

Literature Cited

Connor, R. T., and Straub, G. J.
1941 Determination of thiamin by the thiochrome reaction. Ind. Eng. Chem. **13**: 380–384.

Hennessy, D. J.
1941 Chemical methods for the determination of vitamin B_1. Ind. Eng. Chem. (Anal. Ed.) **13**: 216–218.

Hennessy, D. J., and Cerecedo, L. R.
1939 The determination of free and phosphorylated thiamin by a modified thiochrome assay. J. Am. Chem. Soc. **61**: 179–183.

Schultz, A. S., Atkin, L., and Frey, C. N.
1937 A fermentation test for vitamin B_1. J. Am. Chem. Soc. **59**: 948–949.
1937 A fermentation test for vitamin B_1–II. J. Am. Chem. Soc. **59**: 2457–2460.

Schultz, A. S., Atkin, L., Frey, C. N., and Williams, R. R.
1941 Application of the sulfite cleavage of thiamin to the yeast fermentation method. J. Am. Chem. Soc. **63**: 632.

THE DISTRIBUTION OF THIAMIN IN THE WHEAT PLANT AT SUCCESSIVE STAGES OF KERNEL DEVELOPMENT [1]

W. F. GEDDES [2] and M. N. LEVINE [3]

(Read at the Annual Meeting, May 1941)[4]

In 1940, a three-year WPA project was initiated cooperatively by the Division of Plant Pathology and Botany and the Division of Agricultural Biochemistry of the University of Minnesota for the purpose of studying the effects of leaf rust (*Puccinia rubigo-vera tritici*) and stem rust (*Puccinia graminis tritici*) on the agronomic properties of spring wheat, the translocation of plant constituents into the developing kernel, and the industrial quality and chemical composition of the resulting grain. This was an extension of a study begun in 1935. The problem has been attacked from two directions, by the so-called inhibitive and the preventive methods of rust control. In the inhibitive series, the rust epidemics were produced in the early stages of plant growth and arrested at given periods by sulfur dusting; in the preventive series, the incidence of rust was excluded by sulfur dusting until certain stages in plant development were reached, after which artificial inoculations were made. Thatcher was used for the leaf-rust and Ceres for the stem-rust experiments.

Current interest in the thiamin content of wheat and the scarcity of information regarding thiamin levels at different stages of maturity warrant the presentation of data on samples from the plots in each series where the incidence of rust was controlled by sulfur dusting throughout the growth and ripening of the plants.

Two dates of planting were employed for the leaf rust studies: April 25 and May 21; and one planting date only, May 21, was used for the study of the effect of stem rust. Sampling of the plots for the chemical studies was begun seven days after blossoming and continued at successive three- and four-day intervals until maturity.

The entire aerial portions of individual tillers were collected, their green weights determined, and the heads clipped off and weighed. The two plant fractions, one comprising the stems and adhering leaves, and the other the heads, were dried at approximately 60°C and their dry weights recorded. The heads were then threshed quantitatively,

[1] Joint contribution from the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Divisions of Plant Pathology and Botany and Agricultural Biochemistry, University of Minnesota. Paper No. 2,001, Scientific Journal series, Minnesota Agricultural Experiment Station. Grateful acknowledgment is hereby made to the personnel of the Work Projects Administration, Official Project No. 165-1-71-124, sponsored by the University of Minnesota, for assistance in carrying out the experimental work.

[2] Professor of Agricultural Biochemistry, Department of Agriculture, University of Minnesota.

[3] Pathologist, U. S. Department of Agriculture, detailed for cooperative work in the Division of Plant Pathology and Botany at the University of Minnesota.

[4] Additional data have been accumulated subsequent to the presentation at the annual meeting and are incorporated in this paper.

and the air-dry weights of the kernels and of the nonkernel portion (glumes and rachis) were determined. In this manner three plant fractions, namely (1) kernels, (2) glumes and rachis, and (3) stems and leaves, were provided for the chemical studies. Since the weights of each fraction and the number of tillers represented were known, it was possible not only to express the results of all analytical determinations on a percentage basis but also to calculate the quantities and percentage distribution of each constituent in the three plant fractions as well as the percentage and total amount in the entire tiller at successive stages of kernel development.

Experimental Results

After each plant fraction was ground in a Wiley laboratory mill to pass the 0.5-mm sieve, thiamin was determined by the thiochrome method, as outlined in *Cereal Laboratory Methods* (4th edition, 1941). The mean results are recorded in Table I, together with data for the moisture content of the entire tillers and the weight per thousand kernels, which serve as indices of the stage of maturity.

The results recorded for the early-sown Thatcher are the means of duplicate determinations on two sets of samples, comprising the controls for both the preventive and inhibitive series but representing identical treatments, while those given for the late-sown Thatcher and Ceres are the means for one set of samples, comprising in each case the controls for the preventive series alone. From the practical standpoint, the data for early-sown Thatcher are of the greatest interest and are diagrammatically represented in Figure 1.

In each test similar trends occurred in the thiamin content of the three plant fractions with progressing maturity. The most important phenomenon elucidated by these studies is the fact that most of the thiamin ultimately found in the plant was present soon after blossoming and that thereafter the thiamin was translocated from the glume and stem fractions into the developing kernels.

On a unit weight basis, expressed as micrograms per gram of dry matter, the thiamin content of the kernels decreased rather consistently during the first few weeks after blossoming and then remained relatively constant, with an apparent tendency to rise slightly at full maturity. The initial relatively rapid decrease may be explained by the fact that the endosperm is low in thiamin and hence, as kernel filling proceeds, the concentration in the entire kernel tends to decrease. Sherwood, Nordgren, and Andrews[5] have shown that the thiamin of the mature wheat kernel is largely concentrated in the germ

[5] R. C. Sherwood, R. Nordgren, and J. S. Andrews: Thiamin in the products of wheat milling and bread, Cereal Chem. 18: 811–819, 1941.

TABLE I

MOISTURE CONTENT, WEIGHT PER THOUSAND KERNELS, THIAMIN CONTENT, AND THIAMIN DISTRIBUTION IN THATCHER WHEAT AT SUCCESSIVE STAGES OF DEVELOPMENT

Date of sampling	Days after blossoming	Weight per 1,000 kernels (dm basis)[1]	Moisture content entire tiller[2]	Thiamin content in micrograms per gram (dm basis)				Total thiamin in micrograms				Distribution of thiamin		
				Kernels	Glumes and rachis	Stems and leaves	Entire tiller	Kernels	Glumes and rachis	Stems and leaves	Entire tiller	Kernels	Glumes and rachis	Stems and leaves
		g	%	μg/g	μg/g	μg/g	μg/g	μg	μg	μg	μg	%	%	%
EARLY-SOWN THATCHER (AVERAGE LEAF RUST = 11%)[3]														
July 8	7	5.4	69.0	7.7	2.8	2.1	2.6	1.2	0.7	3.0	4.9	24.7	15.0	60.3
12	11	9.7	67.2	7.4	2.2	2.0	2.9	2.4	0.6	3.0	6.0	39.9	10.3	49.8
15	14	12.7	65.2	6.0	1.9	1.8	2.7	2.7	0.5	2.8	6.0	44.8	9.0	46.2
19	18	17.0	62.0	5.6	1.5	1.6	2.6	3.3	0.4	2.4	6.1	53.9	7.2	38.9
22	21	23.4	55.8	6.1	1.0	1.5	3.0	4.8	0.3	2.0	7.1	67.3	3.9	28.8
25	24	26.0	52.0	6.2	0.8	1.4	3.0	5.1	0.2	1.8	7.0	71.9	3.1	25.0
29	28	26.6	47.8	6.3	0.6	1.3	2.9	4.6	0.2	1.5	6.3	74.2	2.5	23.3
Aug. 1	31	26.7	43.2	6.5	0.5	1.2	2.9	4.5	0.1	1.2	5.8	77.4	2.0	20.6
LATE-SOWN THATCHER (AVERAGE LEAF RUST = 16%)[3]														
July 22	7	3.4	68.6	7.2	3.3	3.2	3.5	0.9	1.1	4.5	6.5	13.6	17.3	69.1
25	10	6.5	68.3	7.6	2.8	3.1	3.6	1.9	1.0	4.2	7.0	26.5	13.7	59.6
29	14	12.7	63.9	6.6	1.7	2.7	3.5	3.4	0.6	3.4	7.3	45.6	7.5	46.9
Aug. 1	17	15.3	62.4	5.9	1.5	2.4	3.3	3.6	0.5	2.9	6.9	51.9	6.6	41.5
5	21	19.2	56.6	5.7	1.4	2.1	3.4	4.3	0.4	2.2	7.0	63.0	5.6	31.4
8	24	20.7	53.8	5.2	1.1	1.8	3.0	3.9	0.3	1.8	6.0	65.3	5.0	29.7
12	28	23.0	46.8	5.2	0.6	1.2	2.8	4.2	0.2	1.1	5.5	76.2	3.1	20.7
LATE-SOWN CERES (AVERAGE STEM RUST = 5%)[3]														
July 22	7	5.6	65.0	8.2	4.0	2.5	3.3	1.4	2.0	3.8	7.2	19.2	27.3	53.5
25	10	9.9	63.6	7.8	2.8	2.2	3.2	3.1	1.3	3.4	7.8	39.3	17.2	43.5
29	14	14.9	60.1	6.5	2.2	1.7	2.9	3.6	0.9	2.1	6.6	53.6	14.2	32.1
Aug. 1	17	18.0	58.2	6.3	1.7	1.5	2.9	4.1	0.7	1.8	6.6	62.0	10.6	27.4
5	21	19.5	48.7	6.6	1.1	1.0	2.9	5.1	0.4	1.2	6.7	75.8	6.7	17.5
8	24	20.8	37.7	6.4	1.0	1.0	2.8	4.8	0.4	1.1	6.3	76.6	6.2	17.2

[1] Mean of duplicate determinations of the weight of 500 air-dry kernels. Moisture determined by drying a ground air-dry sample to constant weight in a vacuum oven at 100°C.

[2] Computed from the percentage of moisture lost on air-drying and the vacuum-oven moisture values for the three plant fractions.

[3] The severity of rust infection was determined with the aid of the "Scale for Estimating Rust" devised by the U.S.D.A. The average infections shown for the early-sown grain were obtained from three semi-weekly readings; those for late-sown grain were based on two such readings.

and in the tissues just beneath the outer bran, that is, in tissues which are laid down early in kernel development.

The progressive decrease in the thiamin concentration in the glume and stem fractions, especially the former, was very striking; thus in mature plants the thiamin concentration of the glume fraction was only 18% to 25% of what it was one week after blossoming, while in the stem fraction the reductions amounted to from 38% to 57% of the earlier figures. In considering the data for the thiamin concentration of the entire tiller, it must be emphasized that errors in the dry-matter weights and thiamin values for the three plant fractions are accumu-

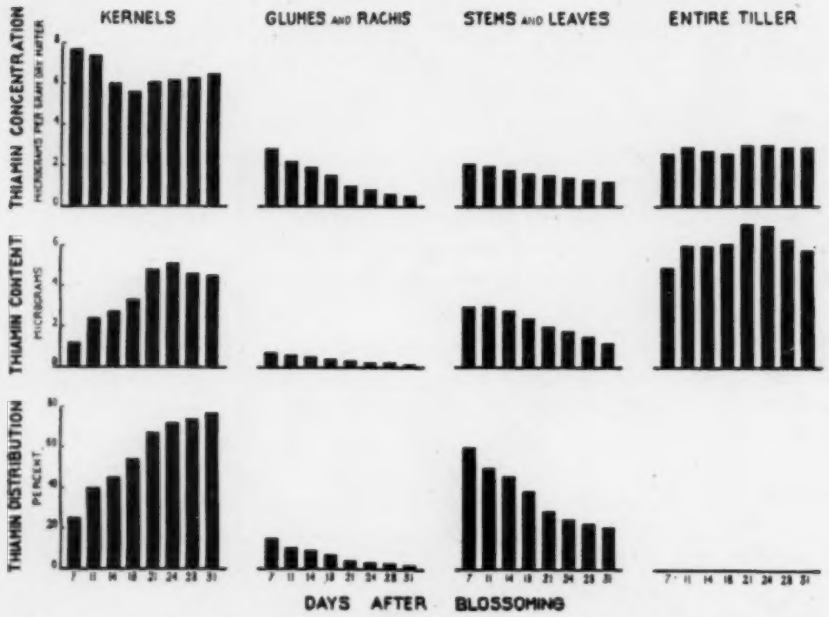

Fig. 1. Diagram showing the concentration, content and distribution of thiamin in different fractions of the wheat plant at successive stages of kernel development.

lated in the computation of the values for the tiller as a whole. In general, however, it would appear that the thiamin concentration of the entire tiller changes only slightly, with progressing maturity; the early-sown Thatcher series showed no definite trend, but with late-sown Thatcher and Ceres there is some indication of a slight decrease as the plant matures.

The thiamin values per unit of dry matter by themselves do not give a complete picture, since the relative weights of the three plant fractions change as the plant matures, the kernels increasing and the glume and stem fractions decreasing in weight. When dry-matter

weights per individual tiller are taken into consideration, that is, when the comparisons are made on the basis of total thiamin, the thiamin content of the kernels increased progressively until the kernel was almost fully matured, after which a slight decrease is noted. This decrease may, however, be only apparent due to loss of kernels through shattering and the depredations of birds. In striking contrast to the kernels, the total thiamin content of the glumes and stems, notably the latter, markedly decreased throughout the entire maturation period.

Considering the data for all three series, the thiamin content of the entire tiller showed no definite trend, fluctuating somewhat in the various sampling periods. As already noted, errors in the thiamin concentrations and dry-matter weights of the three fractions are accumulated in computing these values. The indicated decrease in the thiamin content of the tiller for the last few sampling periods was due, in part at least, to some loss of leaves and also of kernels from the semi-ripe plants. However, the data do indicate that the bulk of the thiamin found in the mature plant is present about one week after blossoming, and as maturity progresses this is translocated from the glume and stem fractions into the kernels.

The translocation of thiamin from the glumes and stems is conspicuously marked by the changes in the distribution of the total thiamin in the three plant fractions with maturity. For example the percentage represented by the kernels in the early-sown Thatcher gradually increased from about 25% at seven days after blossoming to 77% at maturity, whereas the percentage in the glume fraction fell from 15% to 2% and in the stem fraction from about 60% to 21%. In the late-sown Thatcher and Ceres the proportion of thiamin in the kernels was initially somewhat lower, apparently as a result of relatively greater immaturity, but the final values for the kernels were very similar in all series. It is of interest to note that with Ceres, a bearded wheat, a much larger percentage of the total thiamin was present in the glumes than was the case with the beardless Thatcher wheat.

It should be emphasized that the data presented above were obtained with wheat plants in which an effort was made to eliminate the effect of leaf and stem rust on the metabolism of the plant through the control of these parasites by sulfur dusting. Analyses now in progress indicate that stem rust in particular markedly decreased the extent of translocation of the thiamin into the kernels, but a detailed consideration of the effect of leaf and stem rust must await the completion of assays of plants with varying severities of rust infection. Studies are also in progress to determine whether the application of sulfur has, in itself, any influence on the thiamin content of the wheat

plant. The data presented at this time may be regarded as an index of the course of events in the normal wheat plant and it is of particular interest that roughly 75% of the total thiamin of the aerial portions of the mature plant is found in the kernels. The complete physiological inventory of thiamin would require analyses of the roots in addition to the aerial portions but such analyses are impractical under field growing conditions.

Summary

Thiamin assays have been carried out by the thiochrome method on three plant fractions (kernels, glumes and rachis, and stems and leaves) prepared from tillers harvested during kernel filling from plots of early-sown Thatcher, late-sown Thatcher, and late-sown Ceres wheats in which leaf and stem rust were controlled by sulfur dusting. In each series, similar and very striking trends occurred in the thiamin concentration and distribution of the total thiamin in the three plant fractions with progressing maturity.

The thiamin concentration in the kernels decreased slightly during the first few weeks after blossoming, and then remained relatively constant with an apparent slight increase at full maturity. Thiamin concentration in the glume and stem fractions markedly decreased with the filling of the kernels; in the mature plant the values for the glume fractions were only 18% to 25% and those for the stem fractions only 38% to 57% of those at 7 days after blossoming. Thiamin concentration in the tillers as a whole showed little change with maturity, although a slight decrease was indicated in the late-sown Thatcher and Ceres samples.

The total thiamin present in each of the plant fractions changed markedly with maturity. Total thiamin in the kernels increased progressively until the kernel was almost fully matured, the total thiamin in the glume and stem fractions greatly decreased throughout the entire ripening period, while that of the tiller showed no definite trend after an initial slight increase between the seventh and tenth days after blossoming.

The bulk of the thiamin found in the mature plant is present within one week after blossoming; as maturation proceeds it is translocated from the glumes, rachis, stems, and leaves into the developing kernels. Thus, in the early-sown Thatcher approximately 25% of the total thiamin of the tiller was in the kernels, 15% in the glume fraction, and 60% in the stem fraction at 7 days after blossoming. In the mature tiller the kernels contained approximately 77% and the glume and stem fractions 2% and 21%, respectively.

COLLABORATIVE STUDY OF THE APPLICABILITY OF MICROBIOLOGICAL AND CHEMICAL METHODS TO THE DETERMINATION OF NIACIN (NICOTINIC ACID) IN CEREAL PRODUCTS

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(Read at the Annual Meeting, May 1942)

On December 13, 1940, the Research Corporation, New York City, called a meeting of a large number of workers in the field of vitamin assay to discuss the applicability of chemical and microbiological methods to the determination of thiamin, nicotinic acid, and riboflavin in cereal products. The meeting was conducted with Dr. R. R. Williams as chairman; the proceedings were recorded and distributed by the Research Corporation.

The necessity for improving the recently reported methods so that they could be used as reliable control measures in the flour and bread enrichment program became apparent. Various committees were selected to study each of the analytical procedures, to plan collaborative studies, and, on the basis of the results submitted, to suggest standard procedures for use. The author was asked to serve as chairman of the Nicotinic Acid Assay Committee.[1]

The study was divided into two parts. First the collaborators were free to select any of the published microbiological and chemical procedures, including whatever personal modifications they cared to adopt. On the basis of the results submitted, collaborative microbiological and chemical methods were then drawn up for use in the second phase of the study. These methods are reproduced in the Appendix to this report.

The microbiological methods depend upon the growth-stimulating properties of nicotinic acid for certain microörganisms in a medium containing all the factors essential for the growth of the microorganism except nicotinic acid. Metabolic acid production as measured titrimetrically serves as an index of growth, and the nicotinic acid content is interpolated from a standard dose-response curve as determined for each series of tests.

[1] The collaborators who participated in the nicotinic acid study, and their respective laboratories, are included in the following list. They were also members of the Committee: E. E. Snell, University of Texas, Austin; V. Cheldelin and R. R. Williams, Summit, N. J.; C. A. Elvehjem and F. M. Strong, University of Wisconsin, Madison; H. A. Waisman, University of Wisconsin, Madison; O. L. Kline, Food and Drug Administration, Washington, D. C.; W. A. Gortner and J. S. Andrews, General Mills, Minneapolis, Minn.; K. Morgareidge, National Oil Products Company, Harrison, N. J.; D. Melnick and B. L. Oser, Food Research Laboratories, Long Island City, N. Y.; H. Isbell and W. H. Sebrell, U. S. Public Health Service, Bethesda, Md.; C. N. Frey, Fleischmann Laboratories, New York, N. Y.; W. L. Sampson, Merck Institute of Therapeutic Research, Rahway, N. J.; E. B. Brown, Anheuser-Busch, Inc., St. Louis, Mo.; H. J. Cannon, Laboratory of Vitamin Technology, Chicago, Ill.; W. A. Perlzweig, Duke University, Durham, N. C.; W. J. Dann, Duke University, Durham, N. C.; R. B. Meckel, American Institute of Baking, Chicago, Ill.

The chemical methods for nicotinic acid involve strong acid hydrolysis of the test substance, or of a properly prepared extract, to convert all the nicotinic acid derivatives to free nicotinic acid, the common denominator. Preparation of the solution for the colorimetric test may be effected either by adsorption of the pigments, leaving the nicotinic acid in the filtrate, or adsorption of the nicotinic acid and its subsequent elution. The test solution is then adjusted to proper pH and three aliquots are taken. The first is simply diluted to volume to give a measure of residual color of the solution. The nicotinic acid in the second aliquot is allowed to react with cyanogen bromide and an aromatic amine to yield a yellow color which is measured in the photoelectric colorimeter. This is converted into absolute units of nicotinic acid by comparing its photometric density with the increment in photometric density of the third aliquot containing a known increment of nicotinic acid. With these procedures the collaborators assayed four samples prepared and distributed by Dr. J. S. Andrews of General Mills, Minneapolis, and Dr. C. N. Frey of the Fleischmann Laboratories, New York.

Sample No. 1 was a whole wheat flour; No. 3, a white flour; No. 8, an air-dried whole wheat bread made from Sample No. 1; and No. 9, a nicotinic-acid-enriched bread made from Sample No. 3 and then air-dried. On the basis of work reported from our laboratory (Melnick, Oser, and Siegel, 1941), indicating no loss of nicotinic acid during the fermentation of the dough and the baking of the bread, it would seem that the values for the air-dried whole wheat bread should be essentially the same as that for the whole wheat flour, the total solids content of both samples being approximately equal. The following calculations indicated the expected increment for nicotinic acid in Sample No. 9 over No. 3 to be 33 μg per gram.

376 mg nicotinic acid added to 25 lbs flour No. 3.
25 lbs flour yield 37.6 lbs bread.
∴ Increment = 10 mg nicotinic acid per lb bread on fresh basis.
Average total solids in bread by air-drying = 70%.
∴ Increment = 14.3 mg nicotinic acid per lb air-dried bread.
300 g flour (in one lb fresh bread)
2% yeast added (40 mg nicotinic acid per lb)
6.00 g yeast per lb bread.
∴ Yeast furnishes 0.55 mg nicotinic acid per lb fresh bread or 0.8 mg nicotinic acid per lb air-dried bread.
Total nicotinic acid increment in sample No. 9 over No. 3 is:
14.3 + 0.8 = 15.1 mg nicotinic acid per lb air-dried bread or 33 μg nicotinic acid per g.

Thus sample No. 9 served the additional purpose of yielding information on the adequacy of the methods for determining how much nicotinic acid had been added.

Eleven collaborators submitted values using microbiological methods of assay (Snell and Wright, 1941; Dorfman, Koser, Horwith, Berkman, and Saunders, 1940). Most of the investigators were not satisfied to follow any single procedure but actually conducted extensive investigations. It is impossible in the present paper to review all the data submitted. Essentially five methods for the extraction of the nicotinic acid were used; these were mild aqueous,[2] exhaustive aqueous,[3] acid,[4] alkaline,[4] and the use of preliminary enzymic digestion[5] of the sample followed by mild aqueous extraction.

Mild aqueous extraction gave the lowest nicotinic acid values. Predigestion of the samples with enzymes gave only slightly higher figures. Maximal values, more than twice those yielded by the mild aqueous extraction procedure, were obtained on the acid or alkaline extracts. The same figures were found despite variations in concentration of the acid or alkali from 0.1 to 2.0 normality. These maximal values were approximated in the analyses of the exhaustive aqueous extracts. In almost every case, regardless of the methods of extraction, the nicotinic acid values for samples 1 and 8 were practically the same, while the nicotinic acid increment of sample No. 9 over No. 3 approximated the theoretical 33 μg per gram.

TABLE I

MICROBIOLOGICAL NICOTINIC ACID ASSAYS ON ALKALINE EXTRACTS

Collaborator	Procedure	Samples			
		No. 1	No. 3	No. 8	No. 9
		μg per gram			
No. 2	Snell-Wright	65	9.9	60	41
No. 3	"	61	9.9	65	47
No. 4	"	—	—	67	45
No. 5	"	56	8.9	60	41
No. 7	"	69	8.3	63	45
No. 8	"	65	9.9	59	44
No. 11	"	—	9.4	57	39
No. 9	"	62	10.5	64	46
No. 9	Dorfman-Koser	71	12.0	61	43
Average		64	9.9	62	43

A consideration of the various reports indicated that good agreement can be expected if a standard procedure based upon alkaline extraction is used. This is supported by the values listed in Table I

[2] Hot aqueous extraction followed by filtration.
[3] The aqueous suspensions were autoclaved at 15 lbs pressure for 30 minutes and the insoluble residues generally reëxtracted with boiling water one or more times.
[4] The acid (H_2SO_4) and alkaline (NaOH) suspensions were autoclaved at 15 lbs pressure for 30 minutes and the neutralized suspension or extract tested.
[5] Enzymic (takadiastase, papain, pancreatin, etc.) digestion at 40° to 50°C, the suspension heated to 100°C, and the clear filtrate tested.

which indicate, despite the use of alkali of varying normality for the extraction, good reproducibility among laboratories.

Of particular interest was the report by Collaborator No. 9, demonstrating good agreement in nicotinic acid values for cereal products by two unrelated microbiological procedures involving use of different basal media and different microörganisms.

Nine collaborators reported assays using chemical methods for the determination of nicotinic acid (Melnick, Oser, and Siegel, 1941; Dann and Handler, 1941; Melnick and Field, 1940a; Bina, Thomas, and Brown, 1941). A summary of the results of the "more reliable" chemical tests is presented in Table II. The values are in fairly good

TABLE II

NICOTINIC ACID VALUES OBTAINED BY THE "MORE RELIABLE" CHEMICAL TESTS

Collaborator	Procedure	Samples			
		No. 1	No. 3	No. 8	No. 9
		μg per gram			
No. 2	Dann-Handler	60	—	62	—
No. 4	Personal modification of Melnick-Field	—	19.8	—	—
No. 6	Melnick-Field	78	15.0	78	56
	Dann-Handler composite				
No. 8	Melnick-Oser-Siegel	62	13.7	63	48
	Melnick-Field	61	11.5	61	43
	Dann-Handler composite				
No. 13	Melnick-Field	64	14.5	64	46
	Dann-Handler composite				
No. 14 and 15	Dann-Handler	74	19.8	69	50
Average		67	15.7	66	49

agreement with each other. The figures for sample No. 8 were essentially the same as those for No. 1 and the nicotinic acid increment in No. 9 over No. 3 approximated the theoretical 33 μg per gram. The figures agree quite well with the microbiological data (Table I), only the values for sample No. 3, the white flour, being appreciably greater.

The results obtained by the Bina-Thomas-Brown (1941) procedure were not listed, the values varying from 25% to 47% of the average figures listed in Table II. The low values may be attributed to the inherent inaccuracy of this chemical method. It has been shown (Melnick, Oser, and Siegel, 1941; Melnick and Field, 1940b) that methods which include addition of the aromatic amine to the blank give erroneously low nicotinic acid values.

On the basis of the reports sent in by the collaborators, standard procedures were drawn up and submitted for trial. The details of

these collaborative methods are described in the Appendix to this paper.

Some collaborators were of the opinion that the ubiquitous, but nutritionally inactive, betaine of nicotinic acid, trigonelline, is converted during alkaline extraction into a compound capable of stimulating the growth of the microörganism. Trigonelline is resistant to acid hydrolysis but can be hydrolyzed by alkali (Melnick and Field, 1940a; Melnick, Robinson, and Field, 1940) under certain conditions to yield a pyridine compound which reacts like nicotinic acid in the chemical tests. In order to determine whether trigonelline in cereal products interferes in the microbiological assay for nicotinic acid, tests

TABLE III

NICOTINIC ACID VALUES USING COLLABORATIVE MICROBIOLOGICAL PROCEDURES [1]

Collaborator	Sample 1 (whole wheat flour)			Sample 3 (white flour)			Dried cow-peas		
	Extraction			Extraction			Extraction		
	In H_2SO_4	In NaOH	Enzyme[2] + aqueous	In H_2SO_4	In NaOH	Enzyme[2] + aqueous	In H_2SO_4	In NaOH	Enzyme[2] + aqueous
	μg per gram			*μg per gram*			*μg per gram*		
1	59	55	30	8.9	8.6	8.0	18	17	22
2	60	65	44	10.6	9.9	8.4	15	15	21
3	71	69	29	11.4	11.4	6.2	31	31	26
6	57	62	—	10.0	10.0	—	34	23	—
7	59	59	—	8.9	8.8	—	29	17	—
8	57	54	29	9.8	8.5	4.1	20	17	25
9	64	62	—	10.0	9.8	—	26	26	—
11	61	55	43	8.9	8.6	6.3	23	24	18
16	—	68	—	—	—	—	—	25	—
Average	61	61	35	9.8	9.5	6.6	25	22	22

[1] Based upon the method of Snell and Wright.
[2] Papain + takadiastase digestion.

were conducted on both acid and alkaline extracts. For the same reason another product, dried cow-pea meal, was included in the collaborative study. This material is relatively rich in trigonelline (Barger, 1914). The results obtained using the suggested extraction procedures are listed in Table III. The following observations are worth emphasis:

In the microbiological method of assay of cereal products, acid extraction yielded the same nicotinic acid figures as alkaline extraction. In the tests conducted with the cow-pea meal, alkaline extraction definitely failed to yield larger values. These tests constitute good proof that trigonelline does not interfere in the microbiological assays. Enzymic digestion of the sample followed by aqueous extraction

yielded significantly smaller values for nicotinic acid in cereal products. The reproducibility of the microbiological method was good in testing the cereal products but poor in the assay of the cow-pea meal.

Attempts were made to shorten the over-all time of the microbiological method by using a 24-hour rather than a 72-hour incubation period. The results were not satisfactory and therefore are not listed in Table III. The quantity of acid produced during the first 24-hour period was considerably less than that formed during the 72-hour period. Also, consistency among the assay levels in any one test was not good.

Five investigators reported results with the collaborative chemical method. Because of the susceptibility of trigonelline to alkaline hydrolysis, only acid hydrolysis of the samples was carried out. The results, listed in Table IV, show fairly good reproducibility.

TABLE IV

NICOTINIC ACID VALUES USING COLLABORATIVE CHEMICAL PROCEDURE

Collaborator	Sample No. 1 (whole wheat flour)	Sample No. 3 (white flour)	Dried cow-peas
		micrograms per gram	
No. 6	73	15.0	29
No. 7	56	11.9	23
No. 8	61	11.5	27
No. 10	53	8.9	21
No. 15	65	10.3	23
Average	62	11.5	25

Additional tests, summarized in Table V, were conducted with samples 1, 3, 8, and 9 by the collaborative chemical method and a number of modifications of it. In all cases good recoveries of added nicotinic acid were obtained. Exhaustive aqueous extraction was found capable of extracting all of the nicotinic acid from these cereal products. It must be remembered that in these experiments the concentrated extracts were subjected to strong acid hydrolysis, whereas in the microbiological method the aqueous extracts were tested directly. In the collaborative method the importance of adjusting the pH of the final test solution to 7.0 is brought out in the tabulated results. Whereas good recoveries of added nicotinic acid were also obtained in the series in which the pH of the final test solutions was adjusted to 4.5, the initial values were somewhat greater, especially in the case of the white flour. It may be that at pH 7.0 the reaction is more specific for nicotinic acid.

Furfural has been found to react with the reagents (Ashford and Clark, 1939) to give a pink color which absorbs light nonspecifically at 420 mμ, the wave length at which the yellow color due to reacted

TABLE V

NICOTINIC ACID VALUES USING COLLABORATIVE CHEMICAL METHOD AND MODIFICATIONS

Procedure	Nicotinic acid	Sample 1 (whole wheat flour)	Sample 3 (white flour)	Sample 8 (whole wheat bread)	Sample 9 (enriched bread)
		micrograms per gram			
Collaborative	Initial[1]	61	12.2	60	41
	Added	70	30.0	70	50
	Total[1]	144	40.0	130	85
Collaborative but on exhaustive aqueous extract concentrated	Initial[1]	61	10.8	62	45
	Added	70	15.0	70	45
	Total[1]	125	25.8	126	85
Collaborative but pH of final test solution = 4.5	Initial[1]	68	22.4	64	47
	Added	70	30.0	70	50
	Total[1]	133	52.0	123	94

[1] Found by chemical analyses.

nicotinic acid absorbs maximally. We have confirmed this observation and investigated it further. Whole wheat flour may contain as much as 8.6% pentosans, most of it localized in the bran (Bailey, 1925). During the initial acid hydrolysis of wheat samples, in the chemical procedure for the determination of nicotinic acid, furfural is formed. The photometric density at 420 mμ due to reacted furfural was found to be only 1/200 of that for nicotinic acid. Moreover, fully 95% of the furfural in the initial hydrolysate is not present in the final test solution. Thus, chemical analyses of the acid-hydrolyzed whole wheat flour (sample No. 1) for both furfural (Stillings and Browning, 1940) and nicotinic acid showed that the maximal error due to the presence of furfural was 7% of the nicotinic acid value. The pentosans do not interfere noticeably in the method involving initial aqueous extraction (Melnick, Oser, and Siegel, 1941), probably because of their relative insolubility. The fact that essentially the same nicotinic acid values are obtained for the four wheat samples following direct acid hydrolysis of the concentrated aqueous extracts (Table V) is additional evidence of the minimal interference of furfural in the collaborative chemical method. Tests made with nicotinic acid and furfural added to solutions buffered[6] at pH 4.5 and 7.0 indicated that interference due to the presence of furfural is 75% greater when the colorimetric tests are conducted at the lower pH, causing significantly higher nicotinic acid values which are obviously in error.

[6] To the phosphate buffer used in the chemical method for the determination of nicotinic acid concentrated sodium hydroxide solution was added to adjust the pH, and the resulting solutions used in the above tests.

A comparison of the values obtained by the collaborative chemical and microbiological assay procedures is presented in Table VI. The

TABLE VI

COMPARISON OF NICOTINIC ACID VALUES USING COLLABORATIVE CHEMICAL AND MICROBIOLOGICAL ASSAY PROCEDURES

No. of collaborators	Procedure	Hydrolysis of sample	Sample 1 (whole wheat flour)	Sample 3 (white flour)	Dried cow-peas
			micrograms per gram		
5	Collaborative chemical	Acid	62 (13)[1]	11.5 (18)[1]	25 (14)[1]
8	Collaborative microbiological	Acid	61 (12)	9.8 (12)	25 (12)
9	(Snell-Wright)	Alkali	61 (14)	9.5 (13)	22 (14)
1	Microbiological	Acid	69 (6)	13.1 (7)	—
1	(Dorfman-Koser)	Alkali	71 (3)	12.0 (4)	—

[1] Average values are listed. The figures in parentheses represent number of individual analyses reported using the above procedures.

values obtained by five collaborators using the chemical method, by nine collaborators using the Snell-Wright microbiological method, and by one collaborator using the Dorfman-Koser microbiological method are listed. Only the results obtained following acid and alkaline hydrolysis of the samples are compared. These indicate that the values are in good agreement with each other, an excellent index of the specificity of all three methods for nicotinic acid. The values for the cereal products by the chemical method fall between those obtained by the two microbiological procedures.

One point still requires further study before a method can be proposed without reservation. It has been pointed out that, in the microbiological assay, mild aqueous extraction, as such or following enzymic digestion of the samples, yields smaller nicotinic acid values than those obtained when the acid or alkaline extracts are tested. In a report from our laboratory (Oser, Melnick, and Siegel, 1941) it was shown that the increase in nicotinic acid following alkaline extraction is due to a hydrolysis of a compound, otherwise not available to the microorganism, and not to any greater efficiency in extraction. The pertinent data are reproduced in Table VII. The tests were conducted on three dietary mixtures. When the aqueous extracts were hydrolyzed with alkali of the same normality used for the direct extraction of the samples, essentially the same values were obtained, the figures being significantly greater than those for the initial aqueous extracts. Since the microbiological method can determine nicotinamide, nicotinuric acid, and the nicotinamide-containing coenzymes without preliminary hydrolysis (Snell and Wright, 1941), the material respon-

TABLE VII

INDICATION THAT ALKALINE HYDROLYSIS RATHER THAN EXTRACTION IS RESPONSIBLE FOR INCREASED NICOTINIC ACID VALUES BY MICROBIOLOGICAL METHOD [1]

Diet No.[2]	Preparation of sample	Nicotinic acid found	Increase in nicotinic acid value
		mg/3000 cal	%
30	(*a*) Pooled *aqueous* extract and washings after autoclaving	10.2	—
	(*b*) Pooled 2*N* NaOH extract and washings after autoclaving	13.0	27
	(*c*) Aqueous extract, as in (*a*) autoclaved at 2*N* alkalinity	13.3	30
31	As (*a*) above	10.0	—
	As (*b*) above	12.3	23
	As (*c*) above	13.4	34
29	As (*a*) above	7.8	—
	As (*c*) above	9.9	27

[1] Procedure of Snell and Wright.
[2] These were composites of foods prepared by R. R. Williams and represented average American diets.

sible for the apparent greater nicotinic acid values is not one of the above naturally occurring nicotinic acid derivatives.

The evidence that cereal products contain this material, presumably a nicotinic acid derivative which becomes available to the microorganism only after hydrolysis, is presented in Table VIII. The values

TABLE VIII

PRESENCE OF AN UNKNOWN NICOTINIC ACID DERIVATIVE IN CEREAL PRODUCTS

Procedure	Sample 1 (whole wheat flour)	Sample 3 (white flour)	Sample 8 (whole wheat flour)	Sample 9 (enriched bread)
	micrograms nicotinic acid per gram			
Mild aqueous extraction	30	5	30	39
Enzyme extraction at pH 4.5	35	7	35	40
Exhaustive aqueous extraction	50	8	50	42
Alkali extraction	62	10	62	43
Acid extraction	61	10	61	45
Aqueous extract subjected to alkali hydrolysis	65	10	65	44
Aqueous extract subjected to acid hydrolysis	—	—	56	—
Incubation of aqueous suspension at pH 8.0	69	9	—	—
Incubation of aqueous suspension at pH 1.0	65	11	—	—
Mild aqueous extract autoclaved	44	—	44	—

The above values are not, for the most part, those obtained by specific analyses but are estimated from the voluminous data reported by collaborators 2, 3, 5, 6, 7, 8, and 9, using the Snell-Wright (1941) microbiological assay procedure.

listed are not, for the most part, those obtained by specific analyses but are estimated from the voluminous data reported by seven of the collaborators. Mild aqueous extraction yielded minimal values for nicotinic acid in the collaborative samples but still was capable of extracting all of the nicotinic acid added to enrich the white bread. Enzymic digestion at pH 4.5, followed by mild aqueous extraction, gave slightly greater values. Exhaustive aqueous extraction was capable of yielding much greater nicotinic acid figures approximating the maximal and constant values yielded by acid and alkaline extraction. That the latter values were due to hydrolysis and not to more efficient extraction was indicated by taking mild aqueous extracts and subjecting them to alkaline and acid hydrolysis and obtaining thereby the maximal nicotinic acid values. The unknown nicotinic acid derivative is exceedingly labile since incubation of the aqueous suspension at 37°C at pH 8.0 or pH 1.0, or autoclaving a mild aqueous extract, is sufficient to convert the compound to microbiologically active nicotinic acid.

It is impossible to state at the present time exactly how much of the total nicotinic acid in a cereal product is the unknown nicotinic acid derivative. Because of the ready hydrolysis of the compound, it is conceivable that some conversion to nicotinic acid occurs even in mild aqueous extracts during the subsequent sterilization (autoclaving) of the test solutions prior to inoculation with the microörganism.

Cheldelin and Williams (1942) have found this unknown nicotinic acid derivative to be present in cereal but not in meat products. Whether or not this compound in the unhydrolyzed form is nutritionally available to the mammalian species is still a moot question. Attempts to separate it from the known biologically active derivatives of nicotinic acid are being made by several of the collaborators in order to determine through biological assay whether or not the compound in the unhydrolyzed form is available. The little evidence, thus far accumulated, suggests that it is. A black-tongue dog assay conducted on a rice bran concentrate gave a value of 1.65 mg of nicotinic acid per gram (Waisman, Mickelsen, McKibbin, and Elvehjem, 1940), while the microbiological assay values were 0.83 mg. prior to and 1.65 mg after alkaline hydrolysis.[7] The material is very readily hydrolyzed. Simple incubation of the whole wheat and white flours at pH 1.0 or 8.0, pH values common to the human gastrointestinal tract, converts the compound to a form available for the growth of the microörganism *L. arabinosus*.[8]

[7] K. Morgareidge, personal communication.
[8] H. Isbell, personal communication.

In any case both chemical and microbiological methods are able to determine added nicotinic acid with a good degree of accuracy. For this reason, despite the unsettled question of the nutritional value of the unknown nicotinic acid derivative in cereal products, both methods should prove valuable for controlling the enrichment of flour and bread with nicotinic acid.

Appendix

COLLABORATIVE PROCEDURES FOR THE PREPARATION OF EXTRACTS FOR THE MICROBIOLOGICAL ASSAY OF CEREAL PRODUCTS FOR NICOTINIC ACID

Alkaline Extraction

For nonenriched white flour: A two-gram sample is suspended in 50 ml of water, 50 ml of 2*N* sodium hydroxide solution are added, and the suspension is autoclaved at 15 lbs pressure for 30 minutes. The hot suspension is centrifuged and washed twice with 50 ml of boiling water. The "extract" and washings are pooled, neutralized with concentrated hydrochloric acid to pH 7.0 (litmus as the external indicator) and the volume brought to 250 ml.[1]

For whole wheat flour: A one-gram sample is extracted (and hydrolyzed) with 100 ml of 1*N* sodium hydroxide. The final volume is brought to 500 ml.[1]

For other products: By taking appropriate-size samples or by proper dilutions, other cereal products such as enriched flour, enriched bread, bran, etc. may be "extracted" to yield solutions in the proper testing range.[1]

Acid Extraction

Sulfuric acid of the same normality may be used for the extraction in place of the above sodium hydroxide. The neutralization is then made with sodium hydroxide.[1]

Enzymic Digestion Followed by Mild Aqueous Extraction

To a two-gram sample 20 ml of 0.1% acetate buffer at pH 4.6 are added. To the suspension a paste containing takadiastase and papain is added to yield 20 mg of each of the enzyme preparations. The digestion is allowed to proceed for 6 to 8 hours at 48° to 50°C under benzene. The material is steamed for 30 minutes to remove the benzene and to destroy the enzymes, filtered and diluted to appropriate volume. The values obtained by the enzymic digestion procedure will be considerably less than those obtained by the two methods above.

Microbiological Assay

The reference curve is evolved according to the published procedure of Snell and Wright (1941) with slight modifications. If charcoal-treated casein hydrolysates are used p-aminobenzoic acid should be added to the medium, 1 microgram per 10 ml (Isbell, 1942). The medium should contain 1% glucose. The inoculated samples are incubated at 37°C for 72 hours.

COLLABORATIVE CHEMICAL METHOD FOR THE DETERMINATION OF NICOTINIC ACID IN CEREAL PRODUCTS

Based upon the procedures of Melnick and Field (1940a, b), Dann and Handler (1941), Cannon [2] and Gortner [3]

Reagents

Cyanogen bromide: Water saturated with bromine at 5° to 10°C is just decolorized in the cold by the addition of a 10% potassium cyanide solution. From 70 to 75 ml of the potassium cyanide solution is used in the titration of 500 ml of bromine water. This reagent, when stored in the refrigerator at about 5°C, will keep almost indefi-

[1] Further studies have shown that it is unnecessary to separate the extract from the insoluble residue. The extracted suspension after neutralization may be tested directly.
[2] H. J. Cannon, personal communication.
[3] W. A. Gortner, personal communication.

nitely (more than 5 months). A much simpler, but satisfactory, procedure involves simple solution of cyanogen bromide crystals (Eastman Kodak Co., Rochester, N. Y.) in cold water to 4 per cent concentration. The crystals should also be stored in the refrigerator.

Aniline solution: Redistilled aniline is dissolved in absolute ethyl alcohol to make 4% solution. When stored in a brown glass bottle at room temperature, this reagent will keep for months.

Standard nicotinic acid solution: 10.0 mg of nicotinic acid per 100 ml of absolute ethyl alcohol. This solution will keep indefinitely.

Buffer solution: [4] Composed of 1960 ml of water, 30 ml of 15% sodium hydroxide solution, 8 ml of phosphoric acid (85%) and 333 ml of absolute ethyl alcohol.

Hydrochloric acid solution: Concentrated, specific gravity about 1.18.

Sulfuric acid solution: 0.20*N*.

Phosphoric acid solution: A 20% solution.

Sodium hydroxide solution: Concentrated (approximately 18 normal) and 0.50*N*.

Phenolphthalein solution: One % in alcohol.

Methyl violet solution: 0.1% in water.

Lead nitrate.

Tertiary potassium phosphate.

Lloyd's reagent.[5]

Hydrolysis, Adsorption, Elution, and Decolorization

A sample containing from 20 to 100 μg of nicotinic acid is weighed into a test tube calibrated at 15.0 ml and 16.5 ml. Ten ml of water and 5 ml of concentrated hydrochloric acid are added. The tube is immersed in a boiling-water bath and the hydrolysis allowed to proceed for 30 to 40 minutes with occasional stirring. The sample is cooled to room temperature and the pH adjusted to 0.5 to 1.0 with 18*N* NaOH (approximately 3 ml are required) using methyl violet as the external indicator.[6] Two and one-half grams of Lloyd's reagent are added and the suspension shaken vigorously for about one minute. After centrifugation, the supernatant liquid is discarded and the residue washed twice with 10 ml of the 0.20*N* sulfuric acid, care being taken to completely disintegrate it by vigorously shaking at each washing. The suspensions are centrifuged and the supernatants discarded. To the washed residue 12 ml of 0.5*N* sodium hydroxide are added and the mixture shaken vigorously for one minute. Water is then added to bring the volume to the 16.5-ml mark. The volume of solution is 15.0 ml. The tube is again shaken and the resultant suspension centrifuged. The eluate is drained as completely as possible into a clean dry test tube containing 0.7 g of finely powdered lead nitrate.

After shaking for one minute the suspension is centrifuged and the decolorized solution transferred to another clean, dry test tube. One drop of phenolphthalein is added, followed by solid potassium phosphate until the color of the solution becomes slightly pink. One to two drops of 20% phosphoric acid solution is added from a capillary pipette until the solution is neutral to litmus, the latter being used as an external indicator. The suspension is centrifuged. The clear supernatant solution is drawn by oral suction through a U-tipped pipette into a test tube, accurately graduated in 0.1-ml quantities to 15 ml.

Color Development, Readings and Calculations

The volume of the collected supernatant solution is noted and then brought to the 10-ml mark. For the colorimetric tests 3-ml portions of the test solution are used. (*a*) To the first sample 7 ml of the alcoholic buffer solution is added. (*b*) To the second aliquot, 6 ml of the cyanogen bromide reagent is added from a burette, followed in exactly 10 minutes with 1 ml of the aniline reagent. The solutions are stirred after the addition of each reagent. (*c*) To the third aliquot 0.1 ml of the standard nicotinic acid solution is added, followed by the reagents as in (*b*).

The yellow color developed in solutions (*b*) and (*c*) is read after five minutes [7] in a photoelectric colorimeter, using a 420-mμ filter. By subtracting from the photo-

[4] With phenolphthalein as the indicator, 7 ml of the solution have a titratable acidity equivalent to 9.4 ml of 0.05*N* NaOH; its pH is 3.3. These values correspond to the titratable acidity and pH of the pooled reagents 6 ml of CNBr plus 1 ml of $C_5H_5NH_2$, determined under conditions the same as those observed in the reaction with nicotinic acid.

[5] A form of hydrated aluminum silicate, obtained from the Eli Lilly & Co., Indianapolis, Ind.

[6] This is conveniently carried out on a spot plate using as a control methyl violet added to 0.2*N* sulfuric acid.

[7] This corresponds to the maximal color intensity. It reaches a maximum in 3 minutes and is constant for the next 5 minutes.

metric density of solution (*b*) that of solution (*a*) due to the color remaining after decolorization, the photometric density of the reacted nicotinic acid is obtained. This is converted into absolute units of nicotinic acid by correlating the increment in photometric density, solution (*c*) minus solution (*b*), with the amount of nicotinic acid added.

In the colorimetric measurements it is necessary to have two center settings, one for evaluating the residual color in the test solution, the other for the color developed by the chemical reaction. The photoelectric colorimeter is set to give a galvanometer reading of 100.0 (zero photometric density) with a solution containing 3 ml of water, and 7 ml of alcoholic buffer solution. Using the resulting center setting (No. 1, galvanometer reading with the test tube or cuvette containing the pure solution now removed), the blank solutions are read in turn to determine the residual color of each of the test solutions (blank). The colorimeter is then set to give a galvanometer reading of 100.0 with a solution containing 3 ml of water, 6 ml of cyanogen bromide reagent, and 1 ml of aniline solution. All the subsequent solutions containing reacted nicotinic acid are read, using the new center setting (No. 2). Galvanometer readings are converted into photometric density by the formula

$$L = 2 - \log G,$$

where L = photometric density and G = galvanometer reading. (Most instruments have a dual scale which allows direct reading in terms of photometric density; others are supplied with a conversion table.)

If the colorimeter cell requires more than 10 ml (and less than 20 ml) of solution, the decolorized, neutralized test solutions are diluted to double volume with water and 6-ml aliquots are used for testing. Double quantities of reagents are also used. If in place of the test-tube type of cell, a flat cuvette is used, the center settings will be greater than 100.0 and therefore will be off the scale. In such cases the instrument is set to give a galvanometer reading of 50.0 instead of 100.0 and all readings are multiplied by 2 to give the correct value for conversion to photometric density.

Typical Calculations

I. Test substance = 1.000 g of whole-wheat flour.

Eight ml of the final neutralized supernatant solution were diluted to 10 ml, and 3-ml aliquots were taken for testing.

Colorimeter cell = test tube
Volume of the solutions to be read = 10 ml
Center-setting (No. 1) = 73.5 galvanometer reading
Center-setting (No. 2) = 89.0 " "
Solution (*a*)
using center-setting (No. 1) = 90.0 glavanometer reading
= 0.046 photometric density
Solution (*b*)
using center-setting (No. 2) = 36.5 galvanometer reading
= 0.438 photometric density
Solution (*c*)
using center-setting (No. 2) = 15.0 galvanometer reading
= 0.824 photometric density

0.438 − 0.046 = 0.392 photometric density due to reacted nicotinic acid
0.824 − 0.438 = 0.386 photometric density due to reacted nicotinic acid
0.392/0.386 × 10 = 10.2 μg of nicotinic acid in 3-ml aliquot of the test solution
10.2 × 10/3 × 15/8 = 63.8 μg of nicotinic acid per gram of whole wheat flour

II. Same test solution as the above, but the colorimeter cell = flat cuvette and volume of solutions to be read = 20 ml. The 10-ml test solution is diluted with water to 20 ml and 6-ml aliquots are taken for the analyses.

Center-setting (No. 1) = 54.0 galvanometer reading (observed)
= 107.0 galvanometer reading (corrected)
Center-setting (No. 2) = 67.0 galvanometer reading (observed)
= 134.0 galvanometer reading (corrected)
Solution (*a*)
using center-setting (No. 1) = 45.0 galvanometer reading (observed)
= 90.0 galvanometer reading (corrected)
= 0.046 photometric density

Solution (*b*)
using center-setting (No. 2) = 18.25 galvanometer reading (observed)
= 36.5 galvanometer reading (corrected)
= 0.438 photometric density

Solution (*c*)
using center-setting (No. 2) = 7.5 galvanometer reading (observed)
= 15.0 galvanometer reading (corrected)
= 0.824 photometric density

0.438 − 0.046 = 0.392 photometric density due to reacted nicotinic acid
0.824 − 0.438 = 0.386 photometric density due to 10 μg of reacted nicotinic acid
0.392/0.386 × 10 = 10.2 μg of nicotinic acid in 6 ml aliquot of the test solution
10.2 × 20/6 × 15/8 = 63.8 μg of nicotinic acid per gram of whole wheat flour

Summary

Microbiological assays conducted on acid and alkaline extracts of cereal products yield reproducible, maximal values for nicotinic acid.

Aqueous extraction, without preliminary enzymic digestion, removes all the nicotinic acid from cereal products but yields, according to microbiological assays, smaller nicotinic acid values than those obtained for the acid or alkaline extracts.

The apparently greater nicotinic acid content of the acid or alkaline extracts is not due to a greater extraction efficiency but to hydrolysis of an unknown compound, which otherwise is unavailable to the microörganism.

The unknown compound, presumably a nicotinic acid derivative, is believed to be biologically available to man but definite proof of this must await biological assay.

The unknown compound is undoubtedly included in the values obtained by chemical assay since all chemical procedures involve preliminary hydrolysis of the samples (or extracts).

Collaborative microbiological and chemical procedures are described and the results of a collaborative study using these methods are presented.

The collaborative procedures yield reproducible values, good recoveries of nicotinic acid, and are specific for the vitamin in the assay of cereal products.

Furfural, produced from pentosans in wheat products, reacts with the reagents in the chemical procedure for the determination of nicotinic acid. However, in the collaborative method, interference due to the presence of furfural in the final test solution is negligible.

No loss of nicotinic acid occurs in the production of bread from whole wheat flour or nicotinic acid-enriched white flour.

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Literature Cited

Ashford, W. R., and Clark, R. H.
1939 Comparison of colorimetric methods for the determination of nicotinic acid. Trans. Roy. Soc. Can. III, **33**: 29–32.
Bailey, C. H.
1925 Chemistry of wheat flour. Am. Chem. Soc. Monograph Series.
Barger, G.
1914 The simpler natural bases. Monographs on Biochemistry. London and N. Y.
Bina, A. F., Thomas, J., and Brown, E. B.
1941 The determination of nicotinic acid in bread and other cereal products. Cereal Chem. **18**: 661–666.
Cheldelin, F., and Williams, R. R.
1942 Comparison of methods of extraction and assay of nicotinic acid from animal and plant tissues. Ind. Eng. Chem. (Anal. Ed.) (in press).
Dann, W. J., and Handler, P.
1941 The quantitative estimation of nicotinic acid in animal tissues. J. Biol. Chem. **140**: 201–213.
Dorfman, A., Koser, S. A., Horwitt, M. K., Berkman, S., and Saunders, F.
1940 Quantitative response of the dysentery bacillus to nicotinamide and related compounds. Proc. Soc. Exp. Biol. Med. **43**: 434–438.
Isbell, H.
1942 Effect of p-aminobenzoic acid on the microbiological assay for nicotinic acid. J. Biol. Chem. **144**: 567–568.
Melnick, D., and Field, H.
1940a Determination of nicotinic acid in biological materials by means of photoelectric colorimetry. J. Biol. Chem. **134**: 1–16.
1940b Chemical determination of nicotinic acid: inhibitory effect of cyanogen bromide upon the aniline side reactions. J. Biol. Chem. **135**: 53–58.
—— Oser, B. L., and Siegel, L.
1941 Chemical determination of nicotinic acid content of flour and bread. Ind. Eng. Chem. (Anal. Ed.) **13**: 879–883.
—— Robinson, W. D., and Field, H., Jr.
1940 Influence of the excretion of other pyridine compounds upon the interpretation of the urinary nicotinic acid values. J. Biol. Chem. **136**: 131–144.
Oser, B. L., Melnick, D., and Siegel, L.
1941 Comparison of microbiological and chemical methods for determination of nicotinic acid with observations on cooking losses. Abstracts, Div. Biol. Chem., Am. Chem. Soc., Atlantic City, N. J.
Snell, E. E., and Wright, L. D.
1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem. **139**: 675–686.
Stillings, R. A., and Browning, B. L.
1940 Photocolorimetric determination of furfural. Ind. Eng. Chem. (Anal. Ed.) **12**: 499–502.
Waisman, H. A., Mickelsen, O., McKibbin, J. M., and Elvehjem, C. A.
1940 Nicotinic acid potency of food materials and certain chemical compounds. J. Nutrition **19**: 483–492.

THE EFFECT OF CERTAIN INGREDIENTS AND VARIATIONS IN MANIPULATIONS ON THE FARINOGRAPH CURVE

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It has seemed of interest to measure the influence of irregular manipulation and the effects of adding varying amounts of materials, commonly employed in commercial baking practice, upon the Brabender farinograph curves that are characteristically obtained with the usually employed mixture of only flour and water. We have found it convenient to employ a system of tabulating these results that is quite contrary in principle and application to the method commonly followed. This plan of measurement has proved over the past two years to be a simple form of expression that can be readily replicated.

We have selected three terms to designate the three measurements employed: The "initial phase" represents in minutes the period from the start of mixing to the point where the upper side of the curve first touches the 500 consistency line. The "period of resistance" represents the period in minutes measured from the point where the upper side of the curve first touches the 500 consistency line to the point where this same upper side of the curve descends to the 500 consistency line. The "factor X" is plotted by taking one-fourth of the "period of resistance" plus the "initial phase."

This method of measurement has been arbitrarily established, but it is readily applicable to any type of curve and with experience gives a definite picture of flour characteristics as established by the Brabender farinograph. At this time we are offering no suggestions as to the interpretative value of any of these designations beyond the statement that no one of the three measurements, as shown in our data, can be accepted as a single index for scoring flour characteristics.

For the purpose of this study we selected four commercially produced northwestern-type patent flours of the 1939 crop, having previously made a similar study on flour types of the 1938 crop. These flours were carefully blended together in sufficient quantity to provide an adequate supply for the series of tests reported. This flour had an ash content of 0.40%, a protein content of 12.8%, and an absorption of 65.8%. These values were calculated to a 13.5% moisture basis.

Figure 1 represents the normal curve characteristics of this flour blend. We obtained an absorption of 65.8%, an "initial phase" of

2.5 minutes, a "period of resistance" of 22.5 minutes, and a "factor X" of 8.1.

Skovholt and Bailey (1932) have shown that temperature differences cause plasticity variations in doughs, depending upon their stiffness. Brabender and Hartkopf (1938) have recommended that a temperature of 86°F be maintained during manipulation of the farino-

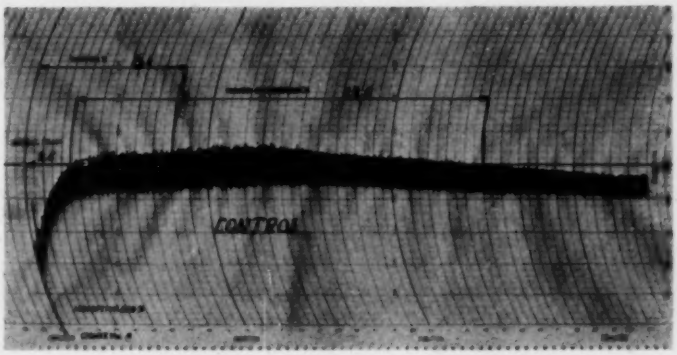

Fig. 1. Normal curve characteristics of flour blend showing plan of measurement employed.

graph. Temperatures employed in commercial bakery practice vary over a considerable range and the Brabender curve obtained at 86°F is by no means comparable with the curve obtained at other temperatures. The effect of varying temperatures is shown in Table I and Figure 2.

TABLE I

EFFECT OF VARYING TEMPERATURES

Temperature	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
76°F	70.6	9.0	18.0	13.5
78°F	69.0	8.0	18.0	12.5
80°F	68.1	7.0	20.0	12.0
82°F	67.2	5.5	19.5	10.4
84°F	66.4	3.5	22.5	9.1
86°F (30°C)[1]	65.8	2.5	22.5	8.1
88°F	64.1	1.5	26.0	8.0
90°F	62.9	1.5	23.0	7.4

[1] Control.

It will be observed that as the temperature increases, the "initial phase" decreased, the "period of resistance" increased, and the "factor X" decreased. The consistency of the dough likewise exhibited a very substantial influence on the characteristics of the curve obtained.

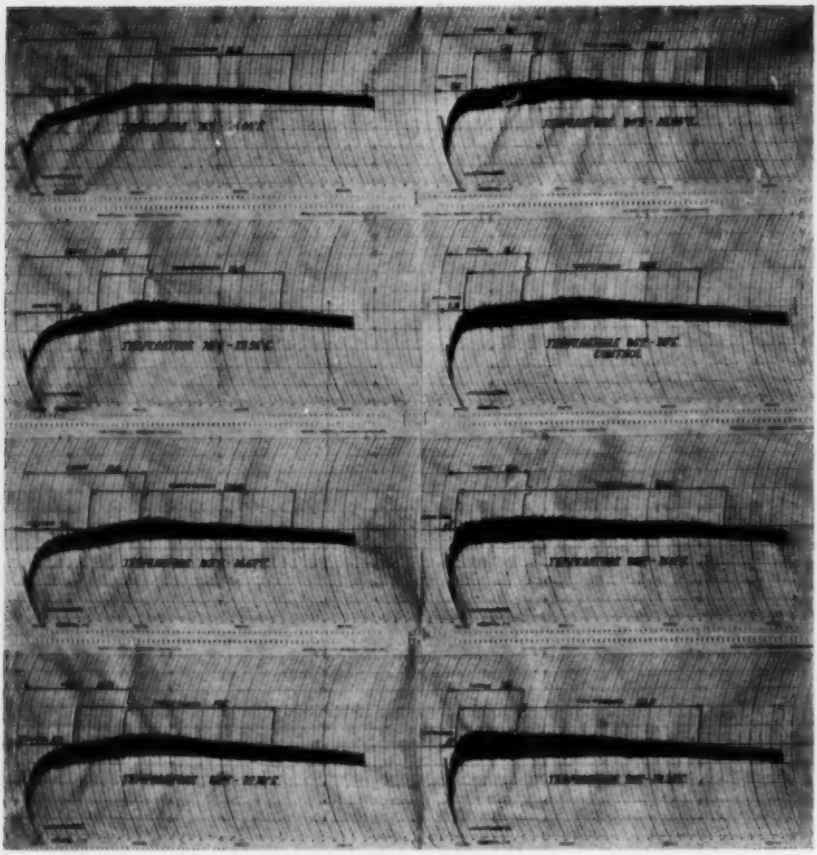

Fig. 2. Effect of varying temperatures.

In Table II and Figure 3 is shown the effect of varying absorptions designed to produce a curve on a definite consistency line, ranging from a normal 500 Brabender units down to 300 units and up to 800 units, in increments of 100 units.

TABLE II

EFFECT OF VARYING DOUGH CONSISTENCY

Units	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
800	56.0	1.0	15.0	4.7
700	58.8	1.0	17.0	5.2
600	61.8	2.5	18.0	7.0
500 [1]	65.8	2.5	22.5	8.1
400	70.1	12.0	28.0	19.0
300	78.9	32.0	22.0	38.2

[1] Control.

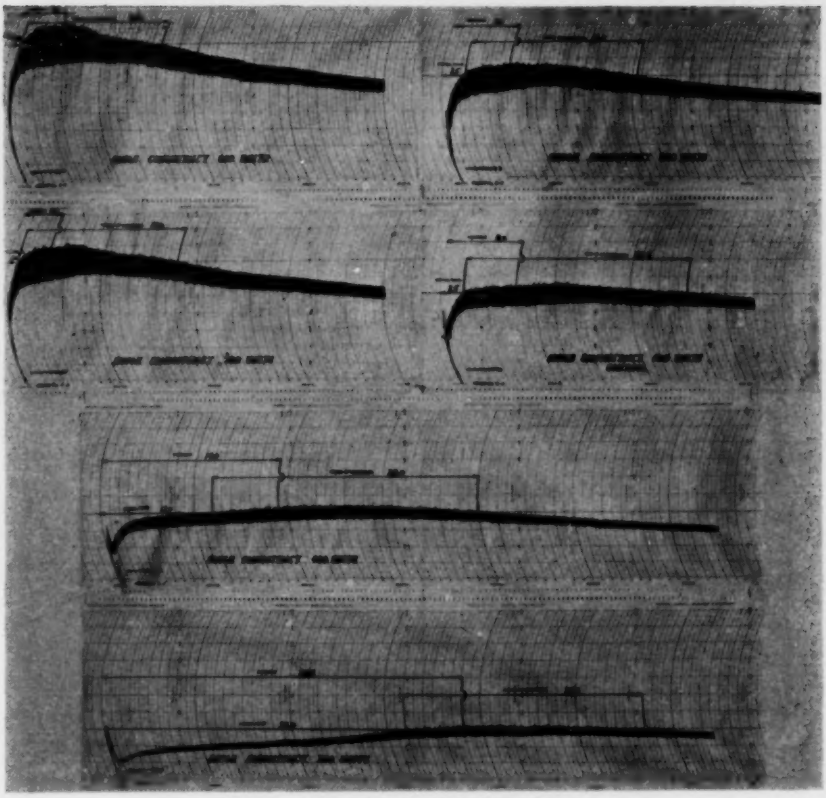

Fig. 3. Effect of varying dough consistency.

It will be noted that the absorption varied from 56.0% for an 800-unit-consistency dough to 78.9% for a 300-unit-consistency dough. The initial phase ranged from 1.0 on the softest dough to 32.0 for the stiffest dough. The period of resistance and factor X increased likewise as the absorption increased.

The effect of salt in the baking process is well established. The addition of varying amounts of sodium chloride to a flour has a marked effect upon the type of curve produced. This is shown in Table III and Figure 4.

TABLE III

EFFECT OF VARYING PERCENTAGES OF SODIUM CHLORIDE

Sodium chloride	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
1%	65.0	5.0	28.0	12.0
2%	65.4	9.0	27.0	15.7
3%	66.5	13.5	29.5	20.0
4%	67.5	18.0	35.0	26.8

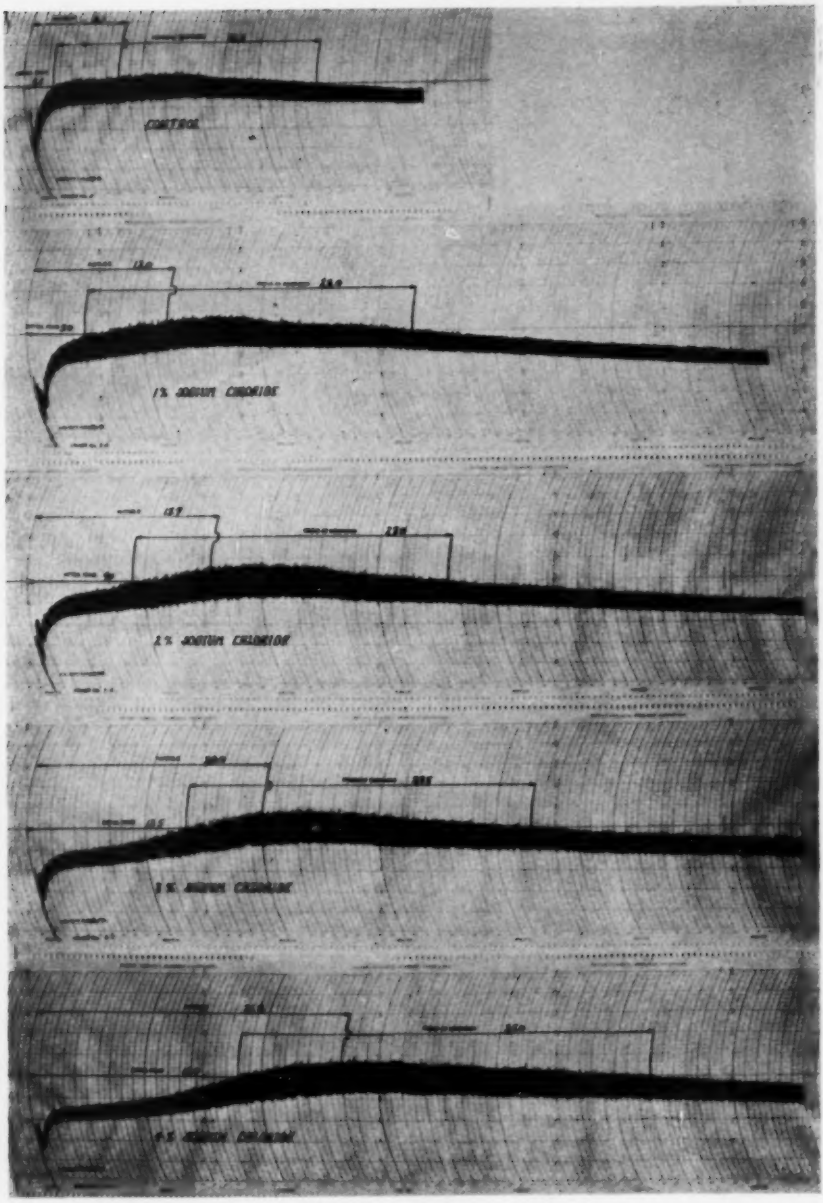

Fig. 4. Effect of varying percentages of sodium chloride.

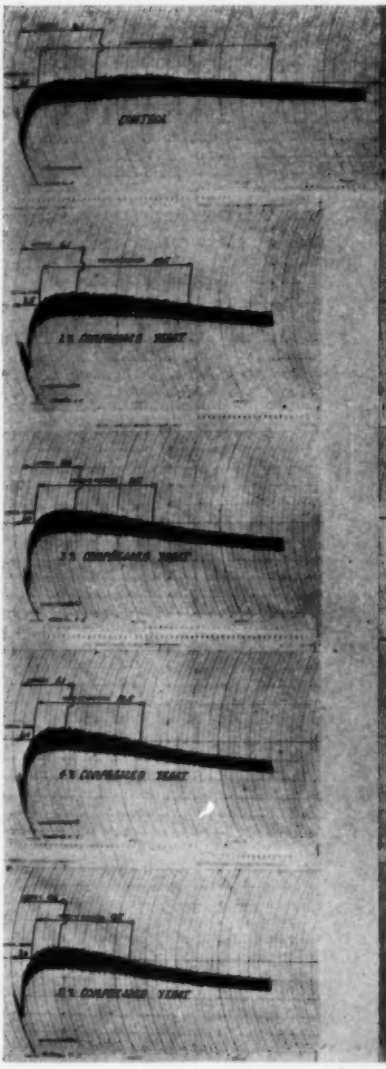

Fig. 5. Effect of varying percentages of compressed yeast.

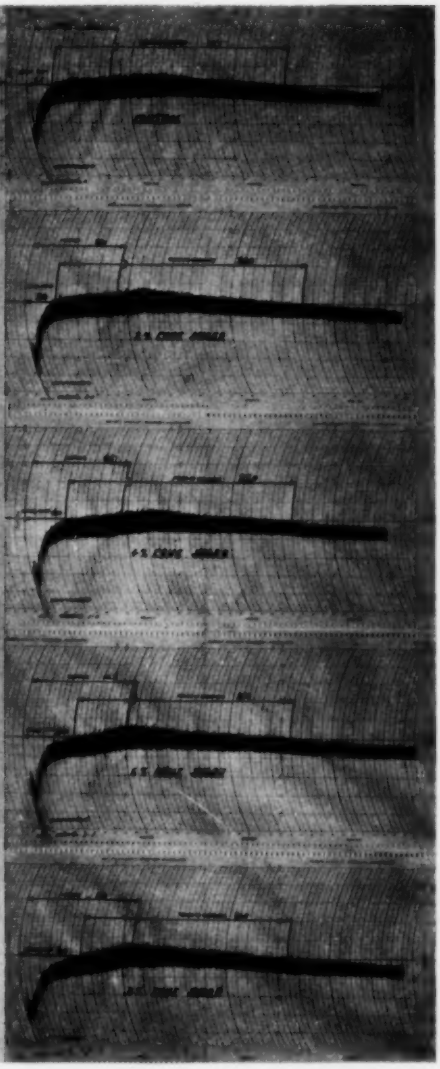

Fig. 6. Effect of varying percentages of cane sugar.

The initial phase, the period of resistance, and the factor X were all increased as the percentage of sodium chloride was increased. A factor X, for example, of 8.1 on the control flour was increased to 26.8 with 4% of sodium chloride. The absorption level increased with the higher increments of sodium chloride.

Compressed yeast when added in different amounts to the control flour had little effect upon either the absorption or the initial phase, but

it did vary the period of resistance and factor X. This influence is shown in Table IV and Figure 5.

TABLE IV

EFFECT OF VARYING PERCENTAGES OF COMPRESSED YEAST

Compressed yeast	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
2%	64.7	2.5	14.5	6.1
3%	64.7	2.0	11.5	5.8
4%	64.7	2.0	10.5	5.1
5%	64.7	2.0	9.5	4.6

Cane sugar likewise had an influence on the characteristics of the Brabender farinograph curve, as indicated in Table V and Figure 6. With increased amounts of cane sugar the initial phase increased, the period of resistance decreased, and the factor X increased.

TABLE V

EFFECT OF VARYING PERCENTAGES OF CANE SUGAR

Cane sugar	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
2%	64.5	3.0	24.0	9.0
4%	64.3	4.0	22.0	9.5
6%	64.0	5.0	21.0	10.2
8%	63.6	6.0	20.0	11.0

Corn sugar had a similar effect on the curve, but the degree of change was more pronounced as indicated in Table VI and Figure 7. It will be observed that 4% of corn sugar raised the initial phase from 2.5 in the control to 7.0, as compared with 4.0 for cane sugar. In turn, factor X was increased to 12.0 from 8.1 in the control and 9.5 for cane sugar.

TABLE VI

EFFECT OF VARYING PERCENTAGES OF CORN SUGAR

Corn sugar	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
2%	65.2	4.0	23.0	9.8
4%	65.4	7.0	20.0	12.0
6%	65.7	7.5	20.5	12.7
8%	65.9	8.0	22.0	13.5

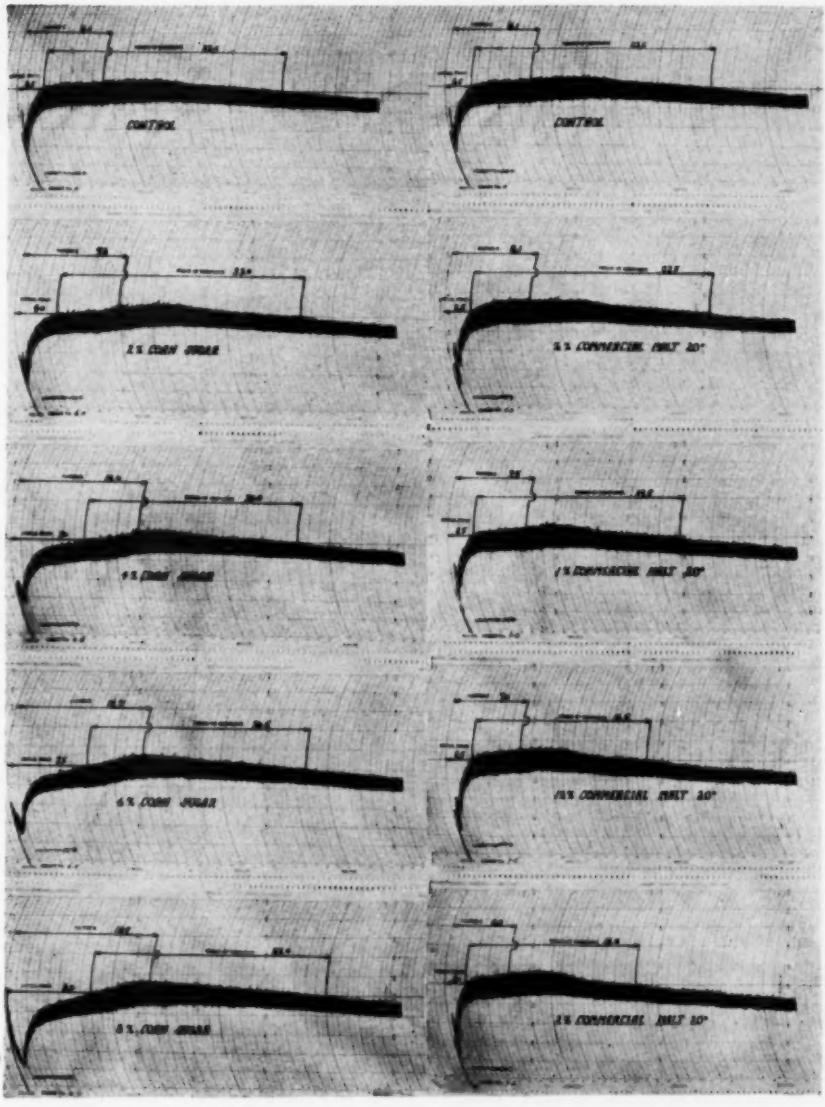

Fig. 7. Effect of varying percentages of corn sugar.

Fig. 8. Effect of varying percentages of commercial malt, 20° Lintner.

The effect of 20°L commercial malt syrup was less pronounced than for either cane sugar or corn sugar. As indicated in Table VII and Figure 8, the period of resistance and factor X showed a slight decrease with increasing increments of 20°L malt added to the flour and water mixture.

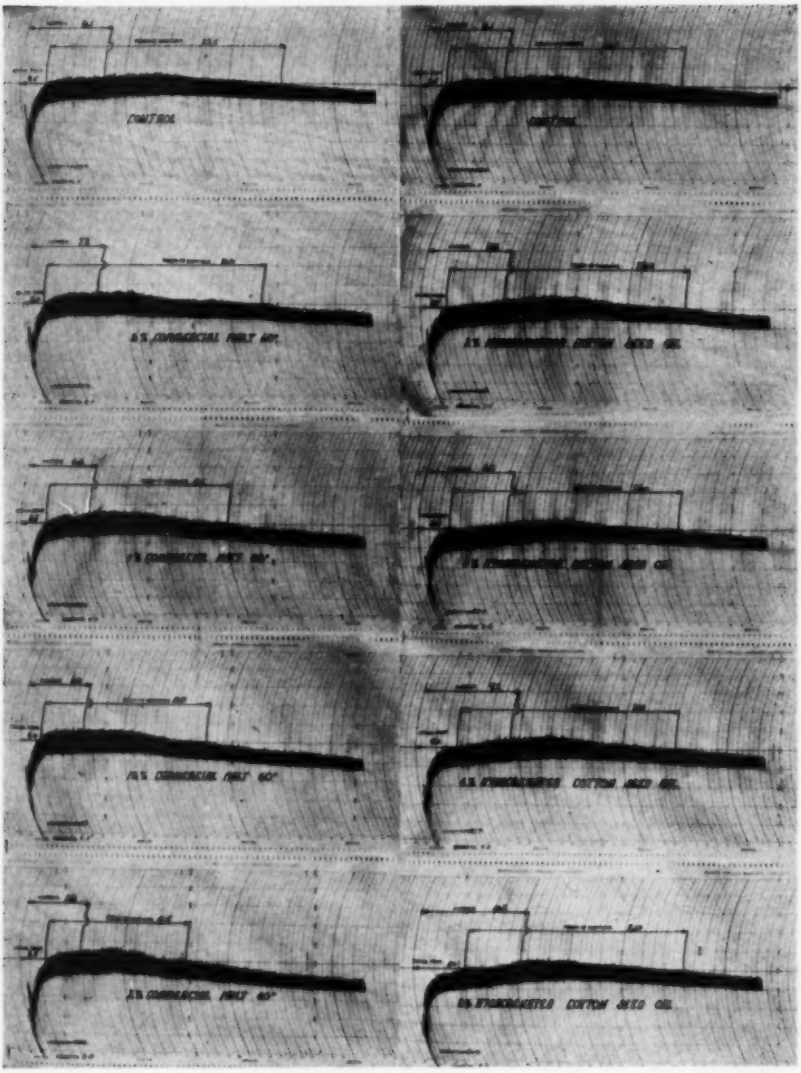

Fig. 9. Effect of varying percentages of commercial malt, 60° Lintner.

Fig. 10. Effect of varying percentages of hydrogenated cottonseed oil.

The degree of variation was slightly more evident with 60°L commercial malt syrup, as shown in Table VIII and Figure 9. In fact, the effect of both types of malt, as would be expected, was quite the reverse of that noted with either cane sugar or corn sugar.

Hydrogenated cottonseed oil in increasing amounts, as indicated in Table IX and Figure 10, raised the initial phase very slightly, like-

TABLE VII

EFFECT OF VARYING PERCENTAGES OF COMMERCIAL MALT, 20° LINTNER

Malt, 20°L	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
½%	64.7	2.5	22.5	8.1
1%	64.4	2.5	19.5	7.5
1½%	63.9	2.5	16.5	7.0
2%	63.6	2.0	16.0	6.0

TABLE VIII

EFFECT OF VARYING PERCENTAGES OF COMMERCIAL MALT, 60° LINTNER

Malt, 60°L	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
½%	64.5	2.0	21.0	7.2
1%	64.2	2.5	17.5	6.6
1½%	63.5	2.0	16.0	6.0
2%	62.8	2.5	13.5	5.8

TABLE IX

EFFECT OF VARYING PERCENTAGES OF HYDROGENATED COTTONSEED OIL

Cottonseed oil	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
2%	63.7	2.5	23.0	8.2
4%	62.9	3.0	22.0	8.5
6%	62.1	4.0	21.0	9.2
8%	62.0	5.0	21.0	10.3

wise the period of resistance, and slightly increased factor X, compared with the control flour.

The effect of dairy butter, as represented in Table X and Figure 11,

TABLE X

EFFECT OF VARYING PERCENTAGES OF DAIRY BUTTER

Dairy butter	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
2%	64.0	2.0	24.0	8.0
4%	63.0	2.0	24.0	8.0
6%	61.2	1.5	26.5	8.1
8%	59.2	1.5	26.0	8.1

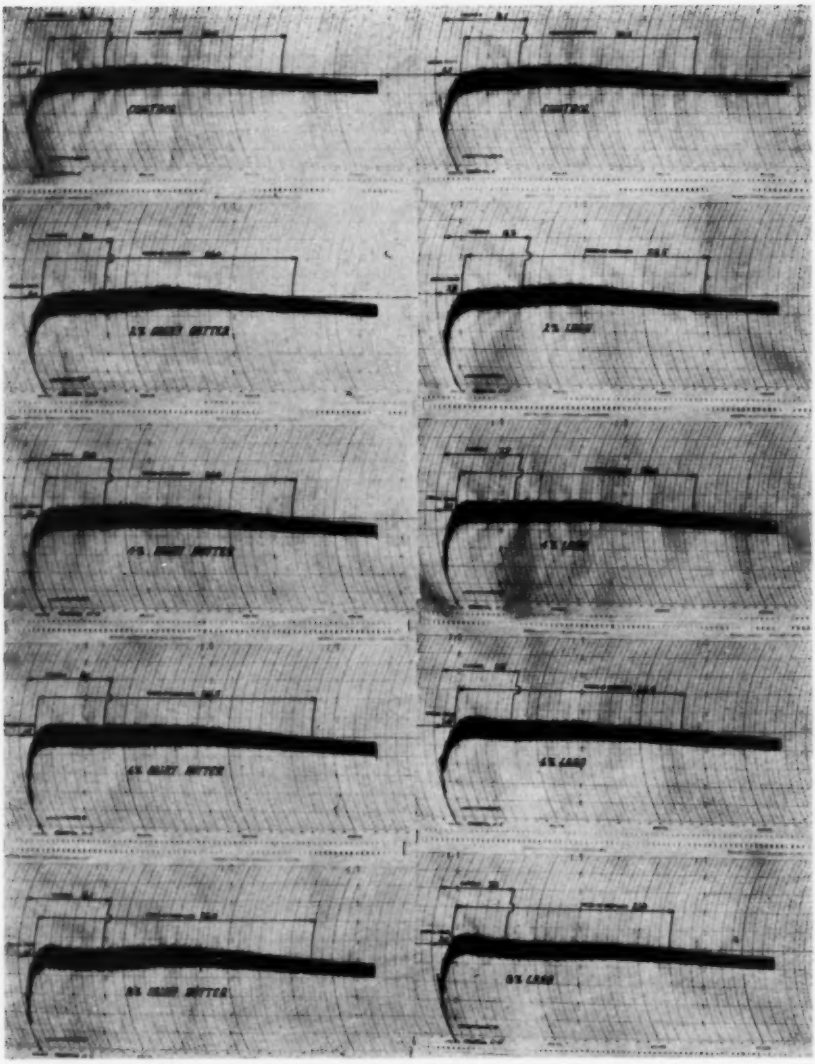

Fig. 11. Effect of varying percentages of dairy butter.

Fig. 12. Effect of varying percentages of leaf lard.

was quite the reverse of the curve characteristics obtained with hydrogenated cottonseed oil. The initial phase was only slightly affected, the factor X was unchanged, and the period of resistance was only slightly advanced with increasing increments of dairy butter. It will be noted, however, that the absorption was lowered from 64% for 2% of dairy butter, to 59.2 when 8% of dairy butter was added to the flour and water mixture.

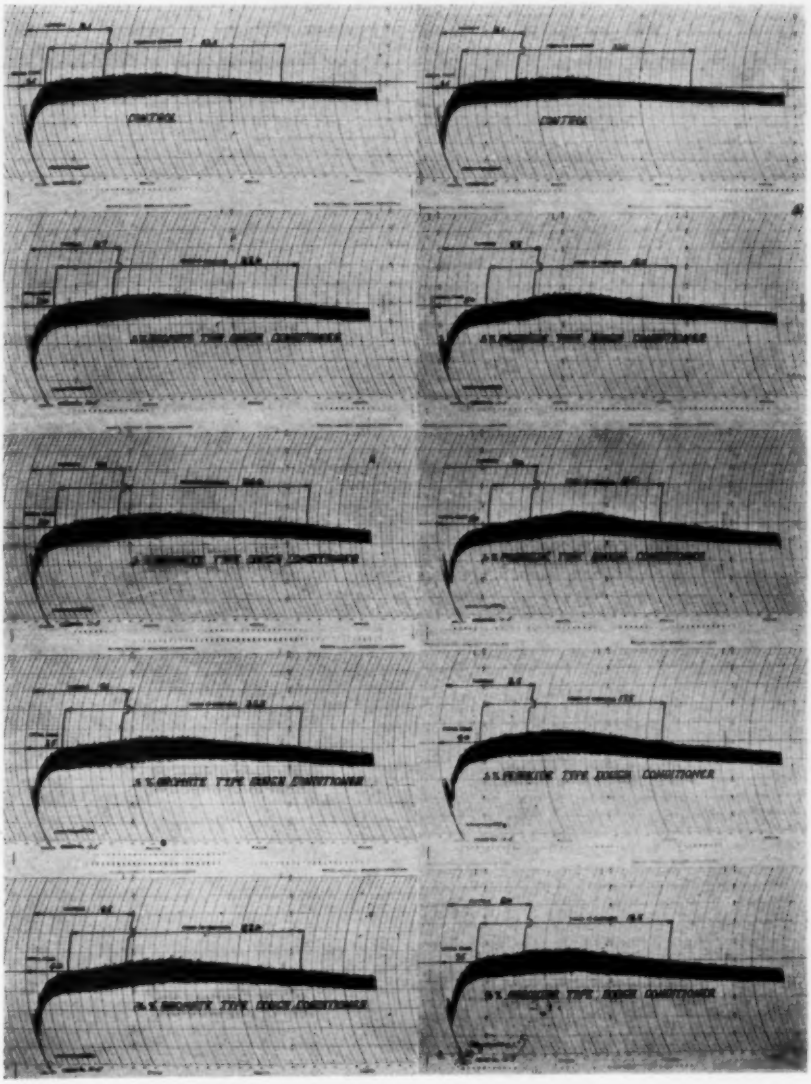

Fig. 13. Effect of varying percentages of bromate-type dough conditioner.

Fig. 14. Effect of varying percentages of peroxide-type dough conditioner.

Leaf lard as shown in Table XI and Figure 12 had much the same reaction as dairy butter. The initial phase and the period of resistance were only very slightly influenced, while "factor X" showed but a minor decrease with increasing increments of leaf lard. The absorption level, however, decreased with increasing amounts of lard in practically the same ratio as with dairy butter.

TABLE XI

EFFECT OF VARYING PERCENTAGES OF LEAF LARD

Leaf lard	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
2%	64.6	2.5	23.5	8.3
4%	63.0	2.0	23.0	7.7
6%	62.2	2.0	22.0	7.5
8%	61.7	2.0	21.0	7.2

Dough conditioners affect the curve to varying degrees. A dough conditioner of the bromate type as represented in Table XII and Figure 13 slightly increased the absorption as the material was added in increasing amounts to the flour-and-water mixture. The initial phase and the factor X were slightly increased in comparison with the control, while the "period of resistance" was only slightly affected.

TABLE XII

EFFECT OF VARYING PERCENTAGES OF BROMATE-TYPE DOUGH CONDITIONER

Bromate conditioner	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
1/8%	65.0	3.0	23.0	8.7
1/4%	65.2	3.0	24.0	9.0
1/2%	65.5	3.5	22.5	9.1
3/4%	65.8	4.0	22.0	9.5

A dough conditioner of the peroxide type, as represented in Table XIII and Figure 14, had a more pronounced effect, particularly in increasing the absorption level. It is interesting to note in this instance that one-eighth of 1% dosage of this material affected the initial phase and the factor X to a greater degree than did larger increments.

TABLE XIII

EFFECT OF VARYING PERCENTAGES OF PEROXIDE-TYPE DOUGH CONDITIONER

Peroxide conditioner	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
1/8%	66.6	5.0	18.0	9.5
1/4%	66.9	5.0	16.5	9.0
1/2%	67.2	4.0	17.5	8.5
3/4%	67.6	3.5	18.5	8.0

A dough conditioner of the iodate and phosphate type, on the other hand, while showing practically no variation in absorption materially

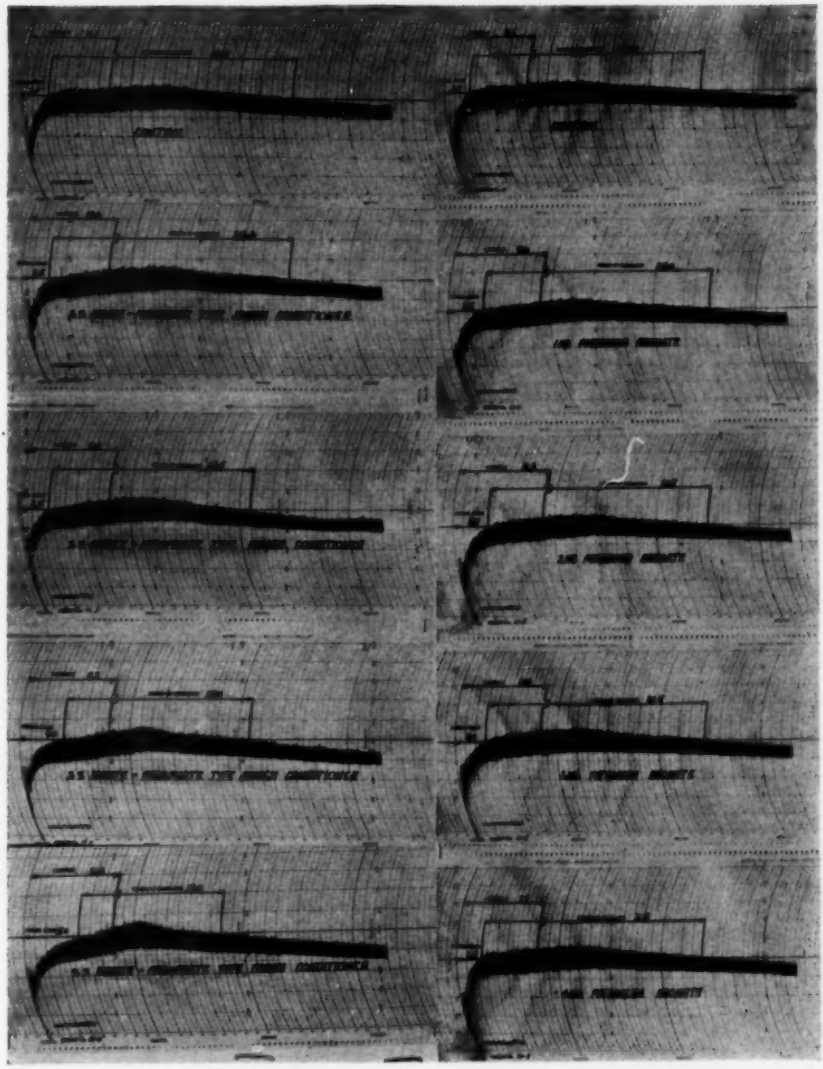

Fig. 15. Effect of varying percentages of iodate-type dough conditioner.

Fig. 16. Effect of varying amounts of potassium bromate.

increased the initial phase and reduced the period of resistance as increasing dosages were added to the flour-and-water mixture. This is shown in Table XIV and Figure 15.

The effect of potassium bromate by itself is not especially different from the results obtained in using the bromate-type dough conditioner. As represented by Table XV and Figure 16, increased increments of potassium bromate had practically no effect upon the absorption level,

TABLE XIV

EFFECT OF VARYING PERCENTAGES OF IODATE- AND PHOSPHATE-TYPE DOUGH CONDITIONER

Conditioner	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
1/8%	65.1	2.5	22.0	8.0
1/4%	65.3	2.5	18.5	8.1
1/2%	65.5	4.0	17.0	8.2
3/4%	65.8	5.0	13.0	8.2

TABLE XV

EFFECT OF VARYING AMOUNTS OF POTASSIUM BROMATE

Potassium bromate	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
1 mg	65.4	3.5	22.0	9.0
2 mg	65.3	3.5	21.5	8.9
3 mg	65.2	3.0	21.5	8.4
4 mg	65.1	2.5	21.5	7.9

while the initial phase, the period of resistance, and the factor X were all slightly decreased.

Skovholt and Bailey (1932) reported on the plasticity characteristics of a dough containing 6% of dry skim milk with and without the further addition of 1.75% of sodium chloride. We have found that dry skim-milk powder, when added to the flour and water mixture, has a pronounced effect upon the "initial phase," the "period of resistance," and the "factor X" as increased percentages of the dry skim milk were employed. This effect is shown in Table XVI and Figure 17.

TABLE XVI

EFFECT OF VARYING PERCENTAGES OF DRY SKIM-MILK POWDER

Dry skim-milk powder	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
2%	65.5	3.5	26.5	10.0
4%	67.1	7.0	24.0	13.0
6%	69.6	10.0	29.0	17.2
8%	71.8	11.5	30.5	19.0

It will be noted, also, that the absorption level increased from 65.5 with 2% of dry skim-milk powder, to 71.8 with 8% of dry skim-milk powder, while the initial phase increased to 11.5 and the period of resistance to 30.5, as compared with the initial phase of 2.5 and the

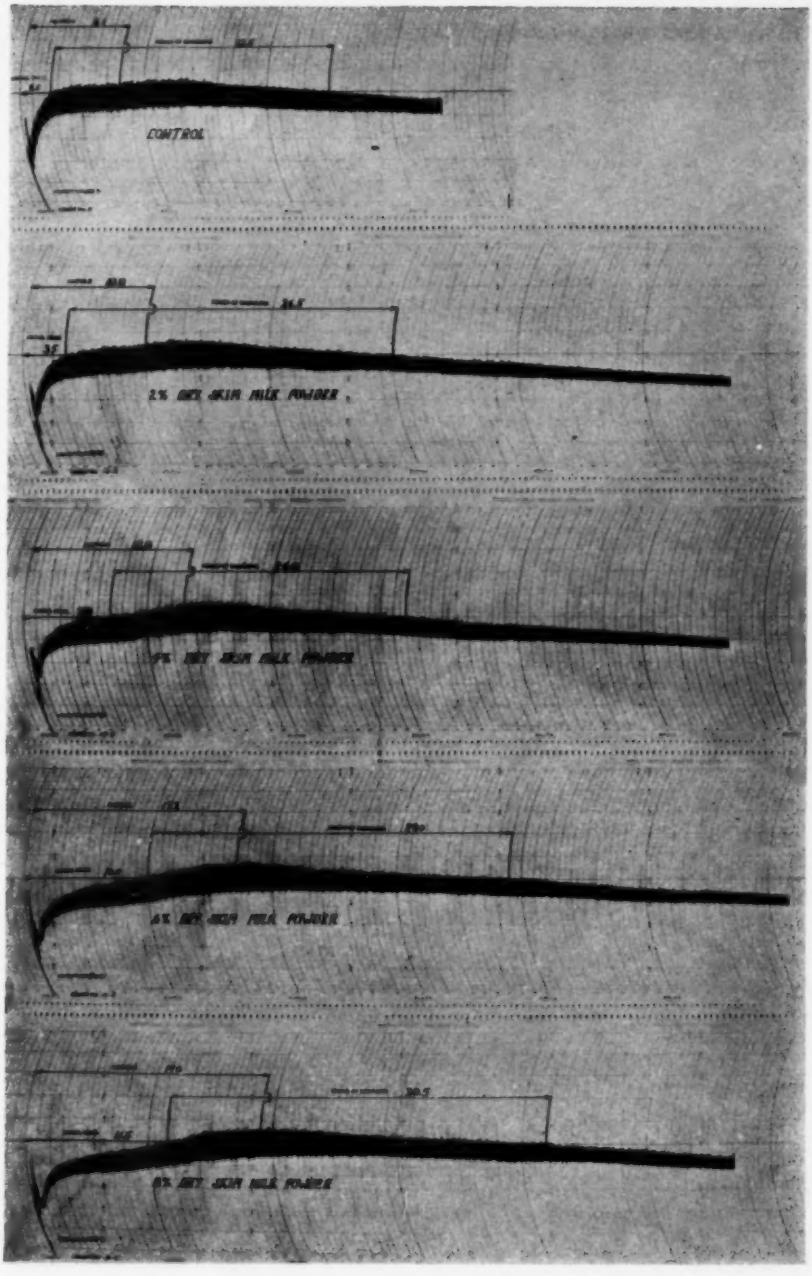

Fig. 17. Effect of varying percentages of dry skim-milk powder.

period of resistance of 22.5 in the control. On the other hand, similar quantities of dry whole-milk powder had a much less pronounced effect, as represented by Table XVII and Figure 18. The absorption level

TABLE XVII

EFFECT OF VARYING PERCENTAGES OF DRY WHOLE-MILK POWDER

Dry whole-milk powder	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
Control	65.8	2.5	22.5	8.1
2%	64.5	2.5	26.5	9.1
4%	64.8	3.5	27.5	10.5
6%	65.5	6.0	25.0	12.2
8%	66.5	8.0	24.5	14.0
EFFECT OF WHOLE LIQUID MILK				
100%	76.5	7.5	9.0	9.7

increased only from 64.5 to 66.5, while the initial phase increased to 8.0 and factor X to 14.0.

For comparative purposes, additional measurements were taken with fresh whole liquid milk in the control flour in place of the water. The absorption obtained with 100% liquid milk was 76.5 as compared with 65.8 for the control, while the initial phase increased to 7.5 in comparison with 2.5 for the control, the period of resistance dropped to 9.0 in comparison with 22.5 for the control, and the factor X increased to 9.7.

The combined effect of certain of these ingredients is clearly shown in Table XVIII and Figure 19. The two curves represented in this

TABLE XVIII

STATEMENT OF LEAN AND RICH FORMULAS

Lean commercial	Dough formulas	Rich commercial
2%	Yeast	2%
¼%	Bromate-type dough conditioner	¼%
½%	20°L malt	½%
2%	Salt	2%
3%	Cane sugar	6%
2%	Hydrogenated cottonseed oil	5%
2%	Dry skim milk	6%

classification cover doughs made from a typical commercial lean formula and a typical commercial rich formula. The formulas employed for these two doughs are tabulated in Table XVIII.

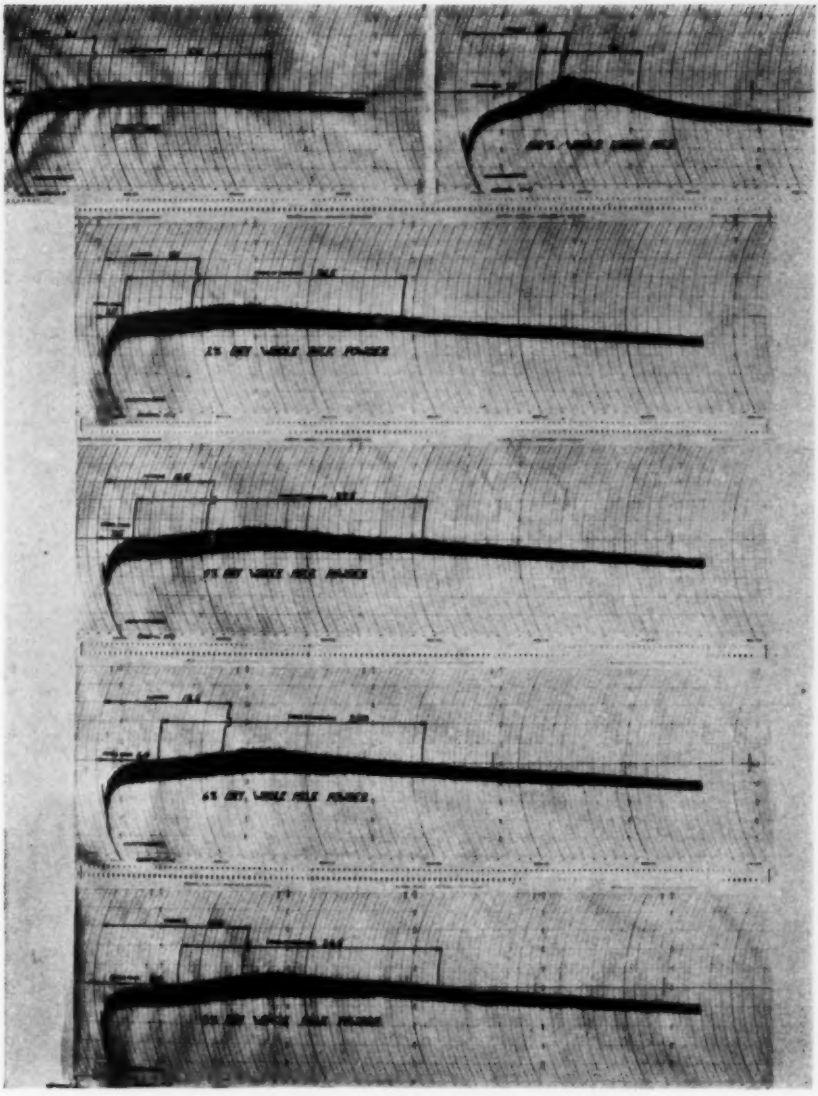

Fig. 18. Effect of varying percentages of dry whole-milk powder.

In comparing the control dough with these two commercial types of bread-dough formulas (Table XIX), it will be observed that the initial phase has been most drastically affected, increasing from 2.5 for the control to 9.0 for the lean formula, and to 17.5 for the rich formula. Factor X has likewise increased from 8.1 for the control to 17.0 for the lean formula and 23.5 for the rich formula.

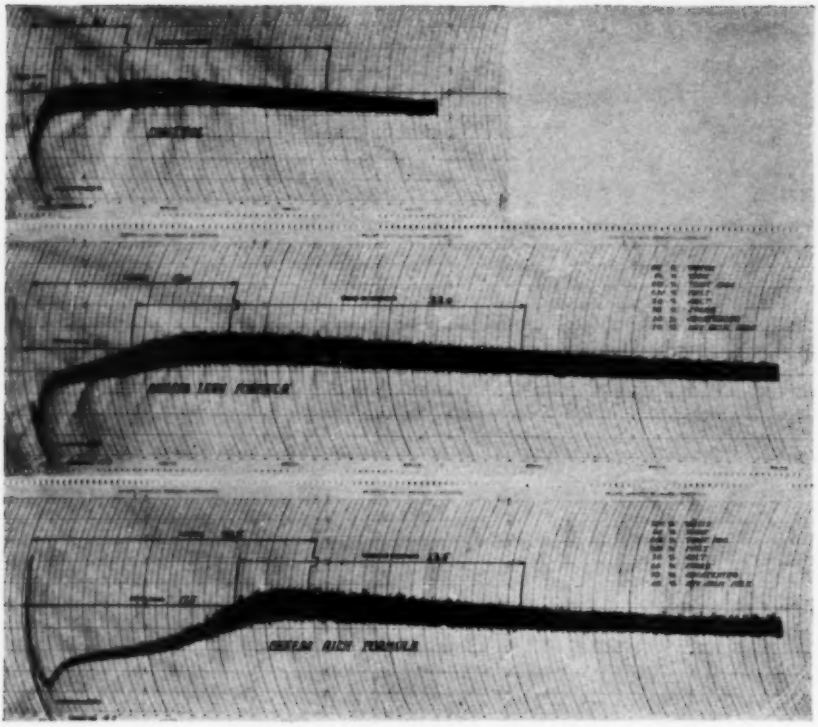

Fig. 19. Effect of lean and rich commercial bread formulas.

TABLE XIX

EFFECT OF LEAN AND RICH FORMULAS

	Percent absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	*min*	*min*	
	EFFECT OF COMMERCIAL LEAN FORMULA			
Control	65.8	2.5	22.5	8.1
	64.7	9.0	32.0	17.0
	EFFECT OF COMMERCIAL RICH FORMULA			
	69.9	17.5	23.5	23.5

Summary

Brabender farinograph curve characteristics are influenced in differing degrees through the addition of varying amounts of materials commonly employed in the commercial baking process. The effects obtained at a maintained temperature of 86°F with sodium chloride, compressed yeast, cane sugar, corn sugar, 20°L commercial malt syrup, 60°L commercial malt syrup, hydrogenated cottonseed oil, dairy butter,

leaf lard, bromate-type dough conditioner, peroxide-type dough conditioner, iodate- and phosphate-type dough conditioners, potassium bromate, dry skim-milk powder, dry whole-milk powder, and whole liquid milk, respectively, are reported.

In addition, the effects of varying temperatures and of differing dough consistencies are shown.

A proposed new system of farinograph curve measurement is suggested.

Acknowledgments

Acknowledgment is made to Roland Smith and Charles Schober for their manipulation of the Brabender farinograph.

Literature Cited

Brabender, C. W., and Hartkopf, Arthur
1938 Personal communications.
Skovholt, O., and Bailey, C. H.
1932 The effect of temperature and of the inclusion of dry skimmed milk upon the properties of doughs as measured with the farinograph. Cereal Chem. **12**: 523–530.

TESTS WITH THE BERLINER-SCHMIDT COLORIMETRIC METHOD FOR THE RAPID DETERMINATION OF SUGARS IN WHEAT FLOUR

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The object of the present paper is to describe a rapid method for determining diastatic activity which has proved very useful in mill control work in Europe. Numerous methods have been proposed for determining the "maltose figure," which is frequently used to control the work of the flour mill. Methods such as those of Rumsey, of Blish and Sandstedt (1933), and of Hildebrand and McClellan (1938) need a fair amount of skill besides numerous chemical solutions. The Kent-Jones (1929) method has been widely used during the last 15 years, but although simpler than the above-mentioned methods, the titration with methylene blue is somewhat difficult. There is a need for a rapid and very simple method for everyday work.

Colorimetric methods for the determination of sugar have involved the use of α-naphthol or picric acid. The latter was used by Blish, Sandstedt, and Platenius (1929) and by Kent-Jones and Saxby (1929). A photocolorimetric method was proposed by Forsee (1938) which requires only one standard solution and is said to be rapid and accurate.

Recently a colorimetric method for determining sucrose using acid sodium selenite has been described by Mohs and Tornow (1940).

The present paper will describe experiments with the Berliner and Schmidt (1933) colorimetric method, which can also be used for the determination of pre-existing sugars (Berliner, 1939). The Blish and Sandstedt (1933) method with the Sandstedt (1937) modification was used for comparison. This method has been subject to some criticism and induced Hildebrand and McClellan (1938) to try a modification using ceric sulfate, which did not seem to give more reliable results. Popoff (1939) also proposed a modification. Klemen (1938) was not satisfied with the results obtained with the Blish and Sandstedt method, which gave results in disagreement with those obtained by some other methods.

Using the Blish-Sandstedt method, Davis (1937) only recovered 88.3% to 93.1% of the theoretical value with pure maltose; Bottomley (1938) obtained 84.0% to 94.1%. However, with the Kent-Jones method the latter recovered 98.8%–100.9% maltose. Blish and Sandstedt (1933) state that the relationship between milligrams of ferricyanide and maltose hydrate is linear; according to Redfern and Johnston (1938) this is true up to 84 mg of maltose.

Method

The Berliner and Schmidt (1933) method is based on the principle that certain sugars caramelize on being boiled in alkali. At first the method was standardized against the method of Berliner and Rüter (1928), not against pure maltose. The figures published in Ziegler's (1940) paper on the Berliner method, although comparative, are not identical with those of the present paper.

The method is now carried out as follows: Digest 10 g of flour with 50 ml of water for 60 minutes at 27°C with continuous shaking. At the end of this time filter (*e.g.* through a Schleicher and Schüll filter paper, No. 588), pouring back the first few drops. After exactly 20 minutes of filtration transfer 15 ml of the solution to a test tube and add 5 ml of approximately normal sodium hydroxide (40 g per liter). Place the test tube in boiling water for exactly 5 minutes. During this time the yellow-colored solution becomes brown, the depth of shade depending on the amount of sugar present. Then cool the test tube, allowing the protein precipitate to settle for 30 to 60 seconds, and measure the depth of color in a colorimeter or compare with standard colors. The color is fairly stable.

Schmidt (1937) stated that protein does not affect the reaction. For comparison he made a series of standard color solutions with bichromate. He states (without giving proof) that the yellow color

given by the water-soluble indicator dyestuff in flour in the presence of alkali is not destroyed by the five minutes of boiling. Simpson (1935) has shown this color to be due to a flavone. We found the greenish tinge in flour extracts disturbing when using the standard colors, which only contain the yellow-brown shade resulting from the caramelization. Berliner [1] has found that this greenish color is due to the presence of sulfur and heavy metals.

Schmidt (1938) has shown that the concentration of alkali can be chosen within wide limits. With concentrations between 0.2*N* and 2*N* no differences were found. He also mentions that the following sugars were caramelized: maltose, glucose, levulose, mannose, arabinose, lactose, galactose, and glycerose. On the other hand the following substances were not caramelized: sucrose, raffinose, starch, dextrin, mannite, and sorbite. He mentions that by caramelization the reducing power of maltose was only partly destroyed. For measuring the maltose figure he advocates the Schmidt and Kühn sugar colorimeter, which embodies a series of colored-glass standards. Berliner and Kranz (1937) advocate the use of a photoelectric colorimeter. The Berliner-Schmidt method is now used in Germany as an official method.

Experimental

For the digestion a rotating thermostat was used with stoppered wide-necked test tubes. Six to 10 test tubes with the filtrate plus alkali were put into a round test-tube holder which was first transferred to the boiling water, then to the table, and finally into the cold water for cooling. The measurement was made in a Buhler photoelectric colorimeter, using a blue filter. This instrument is designed to equalize current disturbances. In the following figures and tables 100% absorption indicates complete darkness and 0% maximum transmittency. The standardization of the instrument was kindly done by Dr. Berliner in Darmstadt. The pure maltose used was Merck's maltose hydrate.

Figure 1 shows the relationship found between maltose and light absorption. Less than 0.25% maltose could not be measured. The usual values in wheat-flour work lie between 1.3% and 3%.

The relationship between Berliner's curve and pure maltose is shown in Figure 2. Since a 20% suspension is used, 15 ml of filtrate contains the maltose formed by 3 g of flour. Thus 30 mg of pure maltose equals 1% maltose. Solutions were made up with pure maltose hydrate in 15 ml of water and treated with 5 ml of alkali as stated above. In Figure 2, curve A is the theoretical curve, whereas curve B is the one actually found. Between 40 mg and 105 mg (1.3%–3.4%), the recovery was 100%. Above and below these limits—*i.e.* outside

[1] Private communication.

the range of practical values in mill control work—the Berliner figures were somewhat low. At the 4% level 98.5% was recovered, at the 5% level 96%. Curve C will be referred to later on.

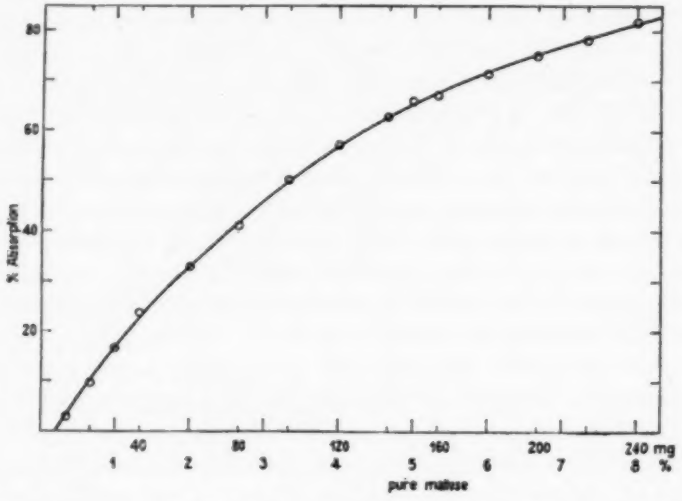

Fig. 1. Light absorption in a photoelectric colorimeter by different amounts of caramelized maltose.

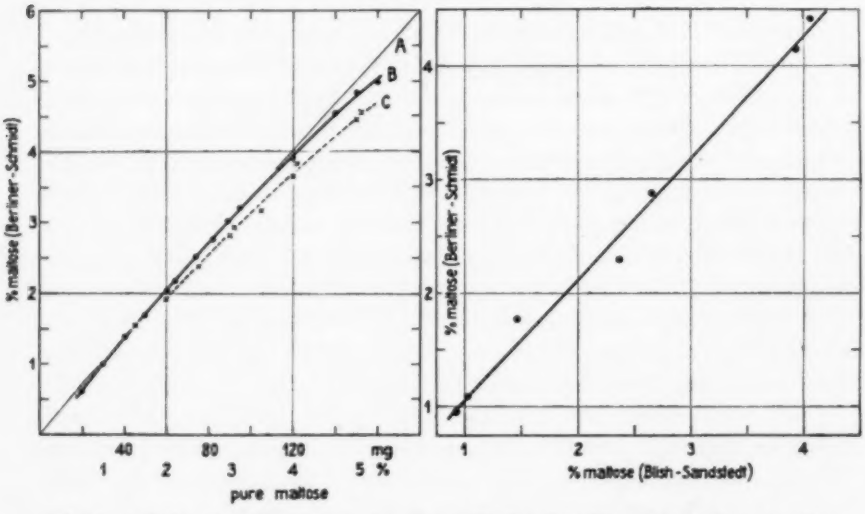

Fig. 2. Relationship between the maltose figure (Berliner-Schmidt method) and pure maltose. A = theoretical curve. B = actual curve. C = maltose recovered after the addition of increasing increments of maltose to flour. Fig. 3 (Right). Comparison of the Blish and Berliner methods.

The Blish-Sandstedt and Berliner-Schmidt methods were compared with each other (Table I). For seven flour samples the difference between extreme values on five different days was 8, 8, 6, 11, 12, 14,

TABLE I

COMPARISON OF TWO METHODS FOR DETERMINING MALTOSE IN FLOUR EXTRACTS

(Single results obtained on five different days)

Flour sample	Blish-Sandstedt method—mg maltose per 10 g flour						Berliner-Schmidt method—% maltose					
	1	2	3	4	5	Mean	1	2	3	4	5	Mean
1	94	90	94	94	94	93	0.92	1.00	0.95	0.97	0.97	0.96
2	104	94	100	100	100	100	1.03	1.11	1.05	1.11	1.11	1.08
3	150	140	150	150	145	147	1.75	1.80	1.74	1.80	1.80	1.78
4	234	227	237	246	237	236	2.30	2.30	2.23	2.34	2.30	2.30
5	266	256	266	266	264	264	2.85	2.93	2.81	2.89	2.93	2.88
6	403	387	396	392	390	394	4.19	4.24	4.10	4.10	4.10	4.15
7	406	400	408	416	406	407	4.50	4.50	4.34	4.39	4.45	4.42

and 16 mg per 10 g of flour for the Blish method and 4, 10, 10, 19, 10, 16, and 16 mg for the Berliner method. The mean values of the seven samples are shown in Figure 3, according to which the Blish values are 94% of the Berliner values, a figure which corresponds with those of Davis (1937) and Bottomley (1938), referred to above.

Table II shows the effect, on the absorption of the water-soluble indicator dyestuff, of the addition of alkali to two flours with widely

TABLE II

COLOR OF THE FILTRATE ON ADDITION OF ALKALI BEFORE AND AFTER BOILING BY THE BERLINER AND SCHMIDT METHOD

	Flour A, 0.49% ash		Flour B, 1.17% ash	
	ABSORPTION, %			
Filtrate alone	2.7		5.5	
" + NaOH (before heating)	4.0	24.0 difference	9.5	16.0 difference
" + NaOH (after heating)	28.0		25.5	
	MALTOSE, %			
Filtrate + NaOH (after heating)	1.67		1.50	

varying ash content but of similar maltose figure. The darkening of color due to caramelization and possibly other transformations is almost twice as great for the low-ash flour as compared with the other sample. This point needs further investigation.

Recovery of Added Maltose

Table III shows that on addition of pure maltose to flour before autolysis, both sugar methods gave only a partial recovery, which was of the same magnitude for both methods, if we take into account that the Blish values are roughly 94% of the Berliner figures. Figure 2,

TABLE III

DETERMINATION OF MALTOSE CONTENT OF A FLOUR WITH ADDED PURE MALTOSE

Maltose added to flour, %	Blish-Sandstedt method (flour A)			Berliner-Schmidt method (flour B)		
	Found	Calculated	Recovery	Found	Calculated	Recovery
	mg maltose per 10 g flour		%	*% maltose*		%
0	175	—	—	1.57	—	—
1	260	275	94	—	—	—
1.5	—	—	—	2.93	3.07	96
2.0	348	375	93	—	—	—
2.5	—	—	—	3.82	4.07	94
3.0	422	475	89	—	—	—
3.5	—	—	—	4.56	5.07	90
4.0	488	575	85	—	—	—

curve C, gives further values for the Berliner method. A few tests were carried out to see where the loss might originate (Table IV). Addition to the flour filtrate gave a 100% recovery (Table IV, Nos. 1–4). Tests on various samples of flour on different days (Nos. 5 and

TABLE IV

RECOVERY OF ADDED MALTOSE

(Maltose determined on 15 ml of filtrate by the Berliner method—recovery given in parentheses)

	Maltose		
	%	%	%
1. 15 ml flour filtrate alone	1.50	—	—
2. 15 ml water + 60 mg maltose	1.94	—	—
3. 15 ml flour filtrate + 60 mg maltose	3.43 (100%)	—	—
4. Calculated value from 1 and 2	3.44	—	—
5. Flour-water suspension (20%) alone	1.50	1.50	1.70
Maltose added	(198 mg)	(350 mg)	(270 mg)
6. Maltose added before autolysis	3.34 (93%)	4.77 (95%)	4.34 (98%)
7. Maltose added after 1 hr autolysis before filtering	3.39	4.77	4.24
8. 81 mg maltose to 15 ml filtrate	—	—	4.24
9. Maltose solution alone	1.98	2.02	2.59
10. + 7 g dried wheat starch	1.94	2.00	2.59
11. + wet gluten from 10 g of flour	1.83 (93.5%)	1.92 (95%)	2.26 (87%)
			Average recovery: 92%

6) gave results similar to those of Table III. On the average the difference was the same whether the maltose was added before or after autolysis (Nos. 6 and 7), but before filtering. To ascertain whether the starch or the gluten was responsible for the loss through adsorption, these two products were taken from 10 g of flour and shaken for one

hour with a maltose solution. The sugar was then determined in 15 ml of filtrate. Whereas the starch had no definite effect, the wet gluten retained about 8% maltose.

Since this method can be carried out even in the simplest of laboratories and needs no great skill it would seem appropriate that collaborative testing of the method be carried out.

Conclusion

The Berliner and Schmidt colorimetric sugar method is most useful for daily control work, because of its simplicity, rapidity, and reliability.

Literature Cited

Berliner, E.
1939 Einige Schnellanalysen für das Mühlenbetriebslaboratorium. Mühlenlab. **9**: 57–64.

—— and Rüter, R.
1928 Titrimetrische und polarimetrische Zuckerbestimmung. Z. ges. Mühlenw. **5**: 116–120.

—— and Schmidt, E.
1933 Über Biuretreaktion, Trommersche Zuckerprobe und eine neue, einfache kolorimetrische Zuckerbestimmung. Mühlenlab. **3**: 187–192.

Blish, M. J., and Sandstedt, R. M.
1933 An improved method for the estimation of flour diastatic value. Cereal Chem. **10**: 189–202.

—— —— and Platenius, H.
1929 Correlation between diastatic power of flour and crust color in the test loaf, and its significance. Cereal Chem. **6**: 121–127.

Bottomley, R. A.
1938 The relationship between diastatic activity and "gassing power" of experimentally milled flours from some Australian wheats. Cereal Chem. **15**: 509–520.

Davis, C. F.
1937 A collaborative study of the Blish-Sandstedt, Schoorl, and Bertrand methods for determining reducing sugars in flour diastatic activity measurements. Cereal Chem. **14**: 81–85.

Forsee, W. T., Jr.
1938 Determination of sugars in plant materials. A photocolorimetric method. Ind. Eng. Chem. (Anal. Ed.) **10**: 411–412.

Hildebrand, F. C., and McClellan, B. A.
1938 An improved method of sugar determination in diastatic activity measurements. Cereal Chem. **15**: 107–113.

Kent-Jones, D. W., and Saxby, J.
1929 Die diastatische Kraft der Mehle. Z. ges. Getreidew. **16**: 171–177.

Klemen, R.
1938 Über die Verwendbarkeit einzelner Eiweissfällungsmittel bei der Zuckerbestimmung in Getreideautolysaten. Biochem. Z. **299**: 63–68.

Mohs, K., and Tornow, Elisabeth
1940 Keimenergie und Selenreduktion. Z. ges. Getreidew. **27**: 17–22.

Popoff, I. D.
1939 Über eine Methode zur Bestimmung der diastatischen Kraft der Getreidemehle und einige Faktoren, welche die diastatische Wirkung beeinflussen. Biochem. Z. **302**: 177–186.

Redfern, S., and Johnston, W. R.
1938 The effect of the substrate on diastatic activity. Cereal Chem. **15**: 327–341.

Sandstedt, R. M.
1937 The adaptation of the ferricyanide maltose method to high diastatic flours. Cereal Chem. **14**: 603–604.

Schmidt, E.
1937 Die Biuretreaktion als mehlchemische Analyse nebst einer schnell ausführbaren Maltosebestimmung. Thesis, Berlin.
1938 Eine kolorimetrische Zuckerbestimmung. Mühlenlab. **8**: 121–132.
Simpson, A. G.
1935 A simple method for determining the "yellowness" and grade of wheat flours. Cereal Chem. **12**: 569–574.
Ziegler, E.
1940 The effect on diastatic activity of surface, pressure, differential and temperature of the reduction rolls in milling. Cereal Chem. **17**: 668–679.

THE ACTION OF HYDROGEN PEROXIDE ON WHEAT FLOUR WHEN USED IN COMBINATION WITH REDUCING AGENTS

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Hydrogen peroxide has been proposed as a dough-leavening agent, either without or in combination with yeast. The production of oxygen gas through the action of flour catalase on the hydrogen peroxide serves as a leavening agent in such methods.

Although hydrogen peroxide is widely used as a bleaching agent (Makow, 1932), it is not commercially used in flour bleaching (Roell, 1934). While Neumann (1929) reports that hydrogen peroxide has a flour-bleaching action, Bailey (1925) states that hydrogen peroxide solution shaken with a benzene extract of flour did not bleach the flour pigments dissolved in the latter.

It has long been known that flour contains the enzyme catalase, which decomposes hydrogen peroxide into water and oxygen. Gelissen (1924) and Van der Lee (1932) show that very small amounts of certain oxidizing agents, such as chlorine (Gelissen) and chlorates, perchlorates, bromates, iodates, periodates, and persulfates (Van der Lee) inhibit the action of catalase on hydrogen peroxide.

Blish and Bode (1935), in a study of catalase activity in wheat flour, report that among flours of comparable grade, but of widely diversified origin, catalase activity varies widely and systematically with the regional or climatic origin of the samples.

The purpose of the present investigation has been to determine whether reducing compounds, such as ascorbic acid, can serve as a means of inhibiting the catalase activity, so that all flours could be brought to the same catalase activity.

Experimental

For purposes of this investigation the absolute value of the catalase activity of the flour used was immaterial, and for most of the experi-

ments an unbleached high-protein straight-grade Texas flour was selected.

Experiments were made according to the method described by Van der Lee (1932): 10 g of wheat flour was thoroughly mixed with 50 ml of water, whereupon 20 ml of an approximately 1.5% solution of hydrogen peroxide was added. In another experiment 20 mg of reducing agent was first dissolved in the 50 ml of water. Both samples were allowed to stand for an hour at room temperature, then filtered rapidly and in aliquot parts of the filtrate the remaining hydrogen peroxide was determined. In a blank experiment the mixture was filtered immediately upon mixing, without standing for an hour, and analyzed. Since in the blank experiment measurable decomposition of hydrogen peroxide occurred during the filtration of the sample, *l*-ascorbic acid was added to retard such decomposition, in order to obtain a more reliable value for the zero time. Some results obtained are given in Table I.

TABLE I

DECOMPOSITION OF HYDROGEN PEROXIDE BY WHEAT FLOUR CATALASE

Duration of catalase action	Material added	Remaining hydrogen peroxide
hrs		%
0	—	100
1	—	38.9
1	*l*-Ascorbic acid	76.9
1	Pyrogallic acid	69.4
1	Gallic acid	77.8
1	Pyrocatechin	77.8
1	Carminic acid	88.9
1	Maleic acid	100
1	Formaldehyde	87.5
1	Sodium hypophosphite	62.9
1	Potassium metabisulfite	85.2
1	Sodium thiosulfate	48.1
1	Ferrous sulfate	76.9
1	Ferrous chloride	81.5
1	Stannous chloride	91.7

Under the conditions of these experiments, when no chemical was added, only 38.9% of the original amount of hydrogen peroxide remained undecomposed after one hour's action of the flour catalase. All the materials used caused considerable retardation of the hydrogen peroxide decomposition.

Similar results have been obtained with urea peroxide, $CO(NH_2)_2$-H_2O_2, instead of hydrogen peroxide, which may be due to the presence of hydrogen peroxide in the urea peroxide solution (Table II).

In the preceding experiments the quantity of reducing agent amounted to 0.2% of the flour weight. It was then appropriate to

TABLE II

DECOMPOSITION OF UREA PEROXIDE BY WHEAT FLOUR CATALASE

Duration of catalase action	Material added	Remaining urea peroxide
hrs		%
0	—	100
1	—	39.0
1	*l*-Ascorbic acid	78.9
1	Gallic acid	87.1

investigate whether smaller amounts of these chemicals have a similar retarding action on the decomposition of hydrogen peroxide by flour catalase. The experiments reported in Table III have been made with

TABLE III

DECOMPOSITION OF HYDROGEN PEROXIDE BY WHEAT FLOUR CATALASE
(With half the concentration of hydrogen peroxide shown in Table I)

Duration of catalase action	*l*-Ascorbic acid in the flour	Remaining hydrogen peroxide
hrs	%	%
0	0	100
1	0	20.3
1	0.2	80.0
1	0.1	70.7
1	0.05	56.3
1	0.01	38.6
1	0.005	32.7
1	0.001	28.2
1	0.0005	19.7

l-ascorbic acid as reducing agent and only half the concentration of hydrogen peroxide used in the experiments of Table I.

With decreasing amounts of reducing agent the inhibiting action on the catalase also decreased, thus providing a means to adjust the catalase activity of different flours to the same level.

Summary

Wheat flour contains catalase which decomposes hydrogen peroxide and thus may prevent bleaching by added hydrogen peroxide. The results show that several reducing agents, when present in small amounts during the action of catalase upon hydrogen peroxide, considerably retard the catalase decomposition of hydrogen peroxide.

Literature Cited

Bailey, C. H.
1925 The chemistry of wheat flour. The Chemical Catalog Company, Inc., New York, N. Y.
Blish, M. J., and Bode, C. E.
1935 Catalase activity in wheat flour. Cereal Chem. 12: 133–142.

Gelissen, H. C. J. H.
1924 Process for bleaching and improving flour and milling products. U. S. Patent 1,483,546.
Makow, V.
1932 F. Ullmann, Enzyklopädie der technischen Chemie, Vol. X. Urban & Schwarzenberg, Berlin, Wien.
Neumann, M. P.
1929 Brotgetreide und Brot. Paul Parey, Berlin.
Roell, L.
1934 Die Backmittel. W. Engelmann, Leipzig.
Van der Lee, G.
1932 Process for enhancing the action of percompounds which are decomposed by catalases upon animal or vegetable products. U. S. Patent 1,866,412.

A METHOD OF DETERMINING DEGREE OF COOKING IN CEREAL PRODUCTS

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The concept of "alkali lability" as applied to starch was developed by Taylor and Salzmann (1933), who recognized two distinct fractions, the alkali-labile and the alkali-stable. The former was quantitatively defined in terms of the amount of alkali taken up by the starch during treatment with hot aqueous alkali. Their practical method was based on the neutralization of the remaining alkali after alkali digestion under a standard set of conditions, followed by iodometric determination of the reducing substances. This method was found to be a tedious one, and gave place to the simplified idea of "alkali numbers" described by Schoch and Jensen (1940). To quote their own words: "Raw starch slowly decomposes in hot aqueous alkali to give simple acidic substances, principally formic, acetic and lactic acids, as well as pyruvic aldehyde. These acidic substances are determined by means of a back-titration technique similar to that used for the saponification of a fat."

Their definition is: Alkali number = ml 0.1*N* NaOH/g starch/1 hour's digestion at 100°C.

Although such a number is an empirical quantity only, and furthermore does not give a direct measurement of aldehyde content, it is of value in following the hydrolytic state of a starch or starchy material. The heterogeneity of raw cornstarch is well shown by Schoch and Jensen, while differences of source material, preparation, and pretreatment for manufacturing purposes are reflected in a wide range of alkali numbers, *viz.* from 6 to 66. The interpretation of these figures on the basis of starch "degradation" awaits further fundamental research, but the practical possibilities of the alkali-number technique are considerable.

Accordingly, it was decided to apply this concept to the assessment of cooking, to see whether a degree of cooking can be estimated by relating it to the alkali number. As far as the writer is aware, there is no ready-to-hand measurement of the degree of cooking, since the very nature of the cooking process is little understood. That the cooking of starch is associated with its gelatinization is obvious, but the extent of chemical, as distinct from physical, change in such a gelatinization process needs to be known. Cooking is not necessarily associated with "dextrinization," which is a loose term to define many unknowns.

Workers in the field have used gelatinization as a criterion (Cunningham, 1931; Hughes, Green, and Campbell, 1938), but this is hardly satisfactory. There is a need, as was stressed 14 years ago by Miner (1928), for a quick means of computing the degree of cooking of cereal products. This paper describes an attempt to develop a procedure that would facilitate control work in all cases where partial or complete cooking of a cereal product is desired during its manufacture.

There are three sections: (1) a critical repetition of Schoch and Jensen's procedure, (2) an investigation into the alkali numbers of various starches, flours, and cereal products as received from mill or manufacturer, and (3) a working experiment on cooking under various conditions, following alkali numbers at each stage.

The Schoch and Jensen Method

The procedure as described by Schoch and Jensen was carried out in detail. Certain modifications were tried, with the results shown in Table I. Each figure represents numerous determinations, all carried

TABLE I

MODIFICATIONS OF THE SCHOCH AND JENSEN PROCEDURE WITH WHEAT STARCH

Conditions	Alkali number	Percent of true value
1. Method as described by Schoch and Jensen	8.3	100
2. Water bath just off the boil	5.0	61
3. Ten ml alkali used instead of 25 ml	3.9	47
4. Larger quantities of starch than the 500 mg specified	5.7	69
5. Volume of total liquid halved	6.7	81
6. Method as described by Schoch and Jensen, but titrate hot	8.3	100
7. No blank calculation	9.1	110

out on one wheat starch, with one variable altered at a time. Of the precautions which these workers observed it was found that only one seemed unnecessary; *viz.*, it did not make any difference whether the bottles were cooled before the titration, provided it was carried out at once.

The following conclusions were drawn from the results shown in Table I (numbers refer to conditions):

2. It is necessary to space the determinations at least five minutes apart, to prevent the water bath from coming off the boil when the bottles are introduced. Accordingly ten determinations may be run in a total working time of two hours.

3. It is necessary to use 25 ml of alkali rather than 10 ml for digestion, even though this necessitates a large quantity of acid for back-titration.

4. In order to decrease percentage error in burette readings, a larger quantity of starch was used. However, this gave low results, so that very accurate burette reading, if possible to 0.02 ml, becomes necessary. It was calculated that with this technique the accuracy of manipulation is such that alkali numbers may be determined to $\pm$ 0.25 unit. By doing each determination in triplicate, an accuracy of 0.1 unit is claimed.

5. Halving the total volume by cutting the water added, but using the same amount of alkali, gives low results also. However, with practice it becomes quite easy to detect a true end point with the larger volume, although in this connection a personal preference was shown for phenolphthalein rather than thymol blue.

6. If back-titration is done at once, the end point is maintained long enough for a reading, so that it becomes unnecessary to spend time cooling the bottles.

7. The blank titer is subtracted from the final titer. In cases where the alkali number is low, the blank is particularly important; for instance a typical titer of 3 ml will have a blank titer of 0.3 ml, which if not accounted for gives a value 10% too high.

Following this survey, which accurately substantiates Schoch and Jensen's precautions, the procedure adopted was standardized as follows:

Starch or starchy material (always sifted or ground, as the case may demand, to pass a 60-mesh sieve) is digested for one hour exactly at 100°C with 25 ml of about 0.4*N* NaOH. Back titration with accurately standardized H_2SO_4 (about 0.2*N*) is done immediately the 60 minutes are up, using phenolphthalein as indicator. In each case triplicate determinations are made, together with a blank in which distilled water is substituted for the alkali.

The alkali number is then represented as follows:

$$\frac{\text{(ml acid for back-titration)} - \text{(ml acid corresponding to blank titration)} \times \text{normality of acid} \times 10}{\text{g of starting material on dry basis.}}$$

Investigation of Various Cereal Products

Using the above technique, triplicate determinations were made on starches, flours, and certain cereal products as obtained from the manufacturer. Table II is arranged in ascending order of alkali numbers.

TABLE II

ALKALI NUMBERS OF VARIOUS CEREAL PRODUCTS

Material	State of material	Alkali number
Corn starch	Raw	7.4
Wheat starch	Raw	8.3
Potato flour	Raw	8.6
Farina	Raw	11.2
Rye flour	Raw	12.7
Buckwheat flour	Raw	14.4
Corn flour	Raw	14.8
Wheat flour (low grade)	Raw	15.3
Rolled oats	Raw	15.7
Oat flour	Raw	16.5
Barley flour	Raw	17.0
Farinaceous breakfast food (1)	Precooked	19.0
Bread (crumb)	Cooked	20.1
Bread (light crust)	Cooked, lightly toasted	20.7
Cold breakfast cereal (1)	Cooked, toasted	21.0
" " " (2)	" "	21.2
" " " (3)	" "	21.6
"Pablum"	Precooked	24.0
Cold breakfast cereal (4)	Cooked, toasted	25.2
Farinaceous breakfast food (2)	Precooked	26.6
Bread—dark crust	Cooked and toasted	27.1
Cold breakfast cereal (5)	Cooked and toasted	27.9
Cold breakfast cereal (6)		29.7
Soluble starch		65.0
Maltose (theoretical value)		84.5
Glucose (theoretical value)		85.2

From the data in Table II the following conclusions can be drawn: (1) Raw starches have the lowest values, and the corresponding flours are higher. (2) All true raw materials in the table have values of 17 or under. (3) All cooked, pretreated, flaked, and toasted products have values of 19 or over. (4) Evidently a certain hydrolysis of material does take place during cooking and especially during the toasting process. (5) The data indicate an increased alkali number of the starch in cooked material, but it must be pointed out that any changes in the hydrolytic state of the protein also influence the figures.

It must be emphasized here that care should be taken over toasted cereals, which contain added material. Whereas the table shows that cooked cereals in general have much higher values than raw flours, these figures are not comparable, owing to the great differences in sources, material, and treatment.

The only real test for the adaptation of this method to assess degree of cooking is to take a given material and cook it in various ways and to various extents. This has been done using farina and a low-grade wheat flour. These experiments are described in the following section.

Alkali Numbers at Various Stages of Cooking

The variables associated with cooking are as follows: (1) fineness of material, (2) percentage of water in material to be cooked, which is in other words the consistency of the material prior to cooking, (3) time, and (4) temperature and pressure.

These have been investigated in turn, and the alkali numbers of the product after the given treatment have been determined in triplicate according to the technique just described. In each table the percentage of increase due to the treatment is given in the last column.

Fineness of material: It is well known that the coarser the granularity of a material, the longer it takes to cook. There is a marked difference in the alkali numbers of a granular product and of the same product ground to flour fineness when submitted to identical cooking procedures.

TABLE III

FINENESS OF MATERIAL AND ALKALI NUMBER

	Alkali number	Percent increase
Farina—raw material, unground, no cooking	11.2	
Farina—raw material unground, gelatinized until thick in 5 ml hot water	16.4	47
Farina—raw material ground to pass 60-mesh sieve, gelatinized until thick in 5 ml hot water	21.2	90

Table III shows that it is necessary before any cooking treatments to grind granular material to a uniform flour fineness to eliminate this variable. This has been done throughout all alkali number estimations.

Percentage of water: The flour–water ratio for optimum cooking was investigated and the results are shown in Tables IV and V. Cooking consisted of simmering gently, with stirring, in small beakers. It is advisable to use a low gas flame and two gauzes.

A lowering of the amount of water as shown in Tables IV and V enhances the cooking of a raw starchy material, the optimum ratio given for a cereal like oatmeal being 1 to 6 or 7. The alkali number changes markedly with the flour: water ratio; the most favorable conditions for farina, 1:6, gave over 100% increase, while 1:7 ratio in the low-grade flour gave 50% increase.

TABLE IV

FLOUR-WATER RATIO AS RELATED TO ALKALI NUMBER—FARINA

	Alkali number	Percent increase
Raw farina	11.2	—
Cooked 2 minutes; flour : water 1 : 20	16.5	47
" " " ; " " 1 : 10	16.4	46
" " " ; " " 1 : 6	16.6	48
Cooked 5 minutes; flour : water 1 : 10	19.0	70
" " " ; " " 1 : 6	22.9	105

TABLE V

FLOUR-WATER RATIO AS RELATED TO ALKALI NUMBER—LOW-GRADE FLOUR

	Alkali number	Percent increase
Raw flour	15.3	—
Cooked 5 minutes; flour : water 1 : 10	17.1	12
" " " ; " " 1 : 9	19.3	26
" " " ; " " 1 : 8	22.3	46
" " " ; " " 1 : 7	23.0	50

Time: We know that there is a point at which a product is what we call "thoroughly cooked," so that further cooking is useless irrespective of time and temperature. At this point, as shown below, the alkali number also reaches a maximum, and it is this apparent correlation which is stressed in this paper.

TABLE VI

TIME OF COOKING AND ALKALI NUMBER—LOW-GRADE FLOUR

	Alkali number	Percent increase
Raw material	15.3	—
Cooked in 1 : 8 ratio for 1 minute	16.6	8
" " " " " 2 minutes	16.3	6
" " " " " 3 "	16.5	8
" " " " " 4 "	19.5	27
" " " " " 5 "	22.3	46
" " " " " 10 "	24.0	58
" " " " " 15 "	23.2	52

Temperature and pressure: The temperature of cooking was raised from 100° to 125°C by carrying out the experiments in a pressure cooker at 17 pounds per square inch.

Neither increase in time nor increase in temperature and pressure (Table VII) raises the alkali number further than a certain critical value. It is evident that, within the experimental difficulties of standardizing a cooking procedure, a completion of cooking is directly associated with a leveling off of the alkali-number increase.

TABLE VII

TEMPERATURE AND PRESSURE—WITH LOW-GRADE FLOUR

	Alkali number	Percent increase
Raw material	15.3	—
1 : 8 ratio 5 minutes ordinary cooking, atmospheric pressure at 100°C	22.3	46
1 : 8 ratio 5 minutes pressure cooking, 17 lbs per sq in at 125°C	22.4	47

A further experiment was carried out to establish the alkali numbers after a low-grade flour was cooked at higher flour : water ratios. These ratios ranged from 1 : 3/5, a 60% absorption dough, through pastes, up to a 1 : 10 ratio, which is a thin porridge.

The various doughs and pastes were made up and submitted all at one time to 10 minutes of pressure cooking. The results are shown in Table VIII.

TABLE VIII

PRESSURE COOKING AND ALKALI NUMBER—LOW-GRADE FLOUR

	Alkali number	Percent increase
Raw material	15.3	—
Ratio 1 : 3/5 consistency of dough	15.5	2
" 1 : 7/10 " " "	18.2	19
" 1 : 3/4 " " "	18.9	23
" 1 : 4/5 " " thin dough	22.4	46
" 1 : 1 " " thick paste	24.3	59
" 1 : 1 1/2 " " paste	24.9	63
" 1 : 2 " " thin paste	22.7	48
" 1 : 7 " " thick porridge	23.0	50
" 1 : 8 " " porridge	22.4	46
" 1 : 9 " " thin porridge	19.3	26
" 1 : 10 " " thin porridge	17.1	12

The 1 : 3/5 dough appeared dry, rubbery, and yellow after this treatment. Increase of water to 1 : 4/5 ratio rendered a softer, whiter mass and there is a striking increase in the alkali number in this range. Increases in alkali number up to 60% are obtained with the pastes, which cook readily, remain white, and become rather fluid in the cooking.

TABLE IX

A PRECOOKED FARINACEOUS FOOD

	Time	Alkali number
As is	—	23.7
Cooked 1 : 8	2 minutes	23.7
Cooked 1 : 8	4 minutes	24.7
Cooked 1 : 8	5 minutes	24.0

On the other hand, when a "precooked" cereal food is submitted to cooking treatment, however drastic, there is no increase in alkali number; this is shown in Table IX.

Discussion

It has been long known that the addition of certain chemical substances helps the gelatinization of starches (Meusel, 1886); that the addition of dilute mineral or weak organic acids brings about starch hydrolysis (Kirchoff, 1811); and that enzymatic digestion of starch with malt, for instance, eventually causes complete hydrolysis to simple sugars (Guérin, 1835).

Cooking as referred to throughout this paper does not include any of these phenomena. The hydrolysis of starch to dextrins and finally to sugars normally takes place in the body and not during cooking. The alkali-number technique does not measure the same thing as the copper-number or iodometric techniques for starch breakdown products. It deals with the very early "depolymerization" of starch, which evidently takes place along with the rupture of the starch granule; this phenomenon may be likened to a type of loosening of the starch structure accompanied by an unknown but very slight amount of degradation.

For the theoretical aspects of starch depolymerization, reference is made to two important sets of papers recently published (Meyer, Brentano, and Bernfeld, 1940; Lampitt, Fuller, and Goldenberg, 1941). No attempt is made to theorize on the changes which we have followed in this paper. The effect of the presence of protein in raising the raw alkali number (*e.g.* cornstarch 7.4, corn flour 14.8) must be taken into account, with the probability that cooking affects the protein as well as the starch. But it is evident that the change consequent upon cooking any starchy material is a real one, and can be followed with a simple practical technique. Values are obtainable for any raw cereal product and for the extent of increase in cooking for the material under consideration. The more precise interpretation must await further work such as Dr. Meyer is undertaking, but at present this method serves as a useful guide in the cooking of cereal foods.

Summary

A method for assessing the degree of cooking of a starch or a starch cereal has been developed, using the principle of alkali lability.

Values for the "alkali number" of several products are given, ranging from 7.4 for raw corn starch to 30 for a toasted cereal.

Complete cooking of a starchy product raises the alkali number—by 60% in a low-grade flour and by 100% in a low-protein farina.

Cooking a precooked cereal has, however, no effect on the alkali number.

Literature Cited

Cunningham, N. T.
1931 The cooking of cereal porridges. Cereal Chem. **8**: 403.
Guérin, V.
1835 Memoire concernant l'action de la diastase sur l'amidon de pomme de terre. Compt. rend. **1**: 81.
Hughes, O., Green, E., and Campbell, L.
1938 The effect of time and temperature on the percentage of gelatinization of wheat and corn starch and of cereals containing those starches. Cereal Chem. **15**: 795.
Kirchoff, G. S. C.
1811 Tekhnologicheski Zhurnal, St. Petersburg **9**: 3–26.
Lampitt, G. H., Fuller, C. H. F., and Goldenberg, N.
1941 The fractionation of wheat starch. J. Soc. Chem. Ind. **60**: 1–304.
Meusel, E.
1886 Untersuchung über die Quell Kraft der Rhodonate. Jahrsberichte 2099 (1886).
Meyer, K. H., Brentano, W., and Bernfeld, P.
1940 The fractionation of starch. Helv. Chim. Act. **23**: 845–884.
Miner, C.
1928 Problems of the nonflour cereal chemist. Cereal Chem. **5**: 70.
Schoch, J. J., and Jensen, C. O.
1940 A simplified alkali lability determination for starch products. Ind. Eng. Chem. (Anal. Ed.) **12**: 531.
Taylor, T. C., and Salzmann, G. M.
1933 The action of aqueous alkali on starches, amyloses, and modified starches. J. Am. Chem. Soc. **55**: 264.

PRECISION OF FLOUR MOISTURE RESULTS BETWEEN AND WITHIN LABORATORIES[1]

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(Read at the Annual Meeting, May, 1942)

The importance of determining the reliability of various routine analytical procedures employed by the cereal chemist has been clearly recognized, as evidenced by the rather extensive list of experimental errors that have been compiled in the fourth edition of *Cereal Laboratory Methods*. Statistical analyses by Treloar and Harris (1928), Treloar (1932), and Davis and Wise (1933) of data obtained in collaborative studies of moisture, protein, and ash determinations have revealed the existence of rather large systematic errors among laboratories. Thus Treloar (1932), in a study of quadruplicate moisture values for one flour determined in 96 laboratories, reported a range of 2.7% in the mean moisture results obtained by different collaborators. The rejection of outlying moisture values, which left a selected group

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of compact distribution, still gave a wide range (0.8%) among laboratories. Davis and Wise (1933), in a study of 2,026 flour-moisture values compiled from collaborators' reports of the Pioneer Section for a five-year period, also found large systematic differences among laboratories; the error distribution of 99% of all the reports covered a moisture range of 0.99%.

It must be emphasized, however, that in both of these studies the moisture methods used were not identical. Davis and Wise (1933) point out that 65% of the collaborators employed the air oven and 35% the vacuum oven, and a wide range of conditions was reported by those using the vacuum oven. Moreover it was pointed out that changes in moisture content in transmitting samples in ointment tins sealed with waterproof tape may have occurred in some instances and contributed to the systematic error.

In contrast to the large systematic errors between laboratories, the precision of the moisture test within laboratories may be regarded as fairly satisfactory. Treloar (1932) found that the average standard error of quadruplicate moisture tests for 96 laboratories was 0.064%, while Davis and Wise (1933) arrived at an average standard error for replicates within laboratories of 0.19%. More recently Eva, Milton, and Geddes (1939) analyzed ten flour samples ranging from 9.7% to 16.6% in moisture content in duplicate by the 130°C air-oven and vacuum-oven methods. The vacuum-oven method gave a significantly higher mean moisture by approximately 0.1% than the air-oven method. The standard errors were 0.02% and 0.05% for the vacuum-oven and air-oven methods, respectively.

In a recent study of the net weight changes and moisture contents of wheat flour at various relative humidities, Anker, Geddes, and Bailey (1942) had occasion to carry out moisture tests by both the vacuum-oven and air-oven methods. As a check on their laboratory findings, the samples were distributed to three commercial laboratories for moisture tests by the air-oven method. These data provide an opportunity for determining the agreement between the vacuum- and air-oven methods and the precision of the air-oven moisture test between and within laboratories. In view of the large systematic errors previously reported in the literature, it seems worth while to record the results of a statistical analysis of these data.

Experimental

The experimental material comprised an 83% patent flour, which yielded 151 samples varying in moisture content from 9.8% to 14.4% as a result of varying the times of exposure in 5-, 10-, and 24 ½-pound sacks to different relative humidities. Four subsamples were taken

from each sack after thorough mixing in a MacLellan batch mixer and placed in air-tight containers. One sample was employed in this laboratory for moisture determinations in triplicate by both the vacuum-oven and 130°C one-hour air-oven methods as described in *Cereal Laboratory Methods* (4th ed., 1941). The remaining three samples were delivered the same day to each of three local commercial mill laboratories which determined and reported their moisture values in duplicate by the 130°C one-hour air-oven method. In most instances the analyses were made the day following sampling. Initially, one of the commercial laboratories used a slightly different technique from the standard air-oven method but 131 of the samples, covering the entire moisture range, were analyzed by the prescribed air-oven method in all four laboratories.

Results

The mean moisture values for each laboratory and method are summarized in Table I, together with the corresponding standard

TABLE I

MEANS AND STANDARD ERRORS FOR MOISTURE DATA

VACUUM AND AIR-OVEN RESULTS FOR ONE LABORATORY—151 SAMPLES		
	Mean %	Standard error (single determination) %
Vacuum-oven method	12.322	0.041
Air-oven method	12.264	0.042

AIR-OVEN RESULTS FOR DIFFERENT LABORATORIES—131 SAMPLES		
Laboratory	Mean %	Standard error (single determination) %
A	12.163	0.051
B	12.082	0.050
C	12.164	0.032
D	12.094	0.040
All	12.126	0.041

errors for single determinations. The mean moisture content of the 151 samples, determined by the vacuum-oven method, was 0.06% higher than that by the air-oven method. This difference was found to be statistically significant and is in line with a similar observation by Eva, Milton, and Geddes (1939). For all practical purposes, however, the agreement between the two methods is very close, and the standard errors are practically identical.

With reference to the air-oven results on 131 samples, a variance analysis of the combined data for all laboratories revealed that the

mean moistures for the four laboratories were significantly different. The interaction between samples and laboratories was also significant. However, the low replicate variance coupled with the large number of degrees of freedom for this variance renders this a very precise test. The range of the laboratory means was only 0.082%. The standard deviation of "within" samples (which includes the variability due to differences between laboratories, the interaction between samples and laboratories, and the replicate error) was 0.093% as compared with a standard deviation of replicates for all laboratories combined of 0.041%. Taking the level of significance as twice the standard error, a difference of 0.2% (2×0.093) between the mean values of two samples analyzed in different laboratories would be significant. However, if the two samples were analyzed in the same laboratory, a difference of 0.1% (2×0.043) would be significant. For practical purposes the precision between and within laboratories is quite satisfactory.

These studies present a much more favorable picture than those reported by Treloar (1932) and Davis and Wise (1933) and show that excellent agreement in air-oven moisture values may be obtained between different laboratories by strict adherence to the prescribed method.

Summary

The vacuum-oven method gave a mean moisture value 0.06% higher than the 130°C one-hour air-oven method in triplicate analyses by one laboratory of 151 flour samples containing from 9.8% to 14.4% moisture. The standard error for each method was 0.04%.

Mean air-oven moisture values of 12.08, 12.09, 12.16, and 12.16% obtained by four laboratories in the analyses of 131 of the above samples were significantly different. For all laboratories combined, the standard deviation for "within" samples was 0.09% as compared with a standard deviation of replicates of 0.04%. Accordingly, a difference of 0.2% in the means of two duplicate determinations of any two samples analyzed in the different laboratories would be significant; if analyzed in the same laboratory a difference of 0.1% would be significant.

These studies show that excellent agreement in air-oven moisture values may be obtained between laboratories by strict adherence to the prescribed method.

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Literature Cited

Anker, C. A., and Geddes, W. F., with Bailey, C. H.
1942 A study of the net weight changes and moisture content of wheat flour at various relative humidities. Cereal Chem. **19**: 128–150.
Davis, C. F., and Wise, M.
1933 A five-year summary of the monthly check sample reports of the Pioneer Section of the A. A. C. C. Cereal Chem. **10**: 203–212.
Eva, W. J., Milton, Nancy, and Geddes, W. F.
1939 Comparative methods of moisture determination with special reference to the Brabender grinder and oven. Cereal Chem. **16**: 460–468.
Treloar, A. E.
1932 The distribution of errors for the determination of protein, moisture, and ash in flour. Cereal Chem. **9**: 449–472.
Treloar, A. E., and Harris, J. Arthur
1928 Criteria of the validity of analytical methods used by cereal chemists. Cereal Chem. **5**: 333–351.

PROTEASE ACTIVITY AND REDUCING MATTER CONTENT OF WHEAT FLOUR DOUGHS IN RELATION TO BAKING BEHAVIOR[1]

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It has been known for many years that certain oxidizing agents, such as potassium bromate and iodate, ammonium persulfate, chlorine, and nitrogen trichloride, have an "improving action" when used in appropriate amounts in the manufacture of yeast-leavened bread. Relatively minute quantities of these oxidizing agents markedly increase the toughness and stability of the dough and the ultimate effects are observed in the loaf in improved volume, loaf shape, crumb grain, and crumb texture. The importance of these substances has led to much research and considerable speculation concerning their mode of action.

From a review of the early literature by Bailey (1925), it would appear that these flour improvers were generally believed to function by stimulating yeast activity. Geddes and Larmour (1933) and Jørgensen (1935, 1935a) have found that potassium bromate does not influence the rate or the amount of carbon dioxide production in bread doughs. More recently Sullivan, Howe, Schmalz, and Astleford (1940) have reported that this improver depresses gas production very slightly but the differences noted were not regarded as of any significance in relation to its baking effects. Investigators in this field are now generally agreed that the oxidizing improvers pro-

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duce their principal effects by some action on the gluten proteins which improves the gas-retaining capacity of the doughs, but there is no unanimity of opinion as to the fundamental nature of the reactions involved.

Of the various oxidizing improvers, potassium bromate has been the most extensively studied. In a review paper dealing with various aspects of the bromate baking test, Geddes and Larmour (1933) point out that this reagent has proved particularly useful in the experimental evaluation of wheat strength. The bromate baking results were more highly correlated with protein content than those obtained by the A. A. C. C. basic formula. However, bromate baking response is only in part conditioned by protein content and is influenced by a number of other factors such as the presence of green, frosted, or immature kernels in the wheat mix, the extent of natural aging, whether or not the sample has been heat treated, and the presence of germ constituents.

The early experiments of Geddes (1930) served to emphasize the role of germ constituents in flour baking behavior. The addition of untreated wheat germ to a highly refined flour produced "green" or underfermented characteristics in the baked loaf. Of particular importance, in the light of current theories, was the observation that the addition of the germ produced soft, sticky doughs, the inferior handling properties of which were most noticeable immediately after mixing and that the harmful effects of the germ on the physical properties of the doughs as well as on the volume, external appearance, and crumb grain and texture of the baked loaves decreased with an extension of the fermentation time. The addition of potassium bromate or heat treatment of the germ reduced its detrimental effects and at the same time shortened the fermentation time necessary to produce a loaf possessing the characteristics of normal fermentation. In view of these observations it was pointed out at that time that the deleterious effects of the germ could not be ascribed to the proteases present in the germ. If increased proteolytic activity were responsible for the harmful effects, the dough-handling properties and loaf characteristics would become progressively worse instead of progressively better as fermentation time was extended.

Since heat treatment of the germ, the use of bromate, and longer fermentation time each resulted in improved baking results and gave supplementary effects when combined, Geddes (1930) suggested that these treatments had, in part at least, a common basis through a similar action on certain germ constituents and that the baking improvement resulting from natural aging was probably the result of similar physical or chemical changes. On the basis of Working's

studies (1924, 1928, 1928a) on the injurious effects of phosphatides on gluten quality and his views that the beneficial effects of natural aging and chemical improvers might be due to changes in the solubility of the phosphatides, Geddes suggested that they might prove to be the constituents involved in the common improvement resulting from heat treatment, oxidizing agents, and extended fermentation. No actual experiments were conducted to determine the nature of the substance or substances involved. However, Saunderson and Winkler in 1930 and 1931 (unpublished, reviewed by Geddes and Larmour, 1933) found that natural aging and partial ether extraction of wheat germ brought about a considerable reduction and acetone extraction a lesser reduction in its harmful baking effects. Heat treatment and extraction with these solvents decreased the lipoid phosphorus content and the evidence, though indirect, pointed to a close relation between phosphatide content and the baking behavior of germ-flour mixtures. Sullivan and Near (1933) found that the lipoid phosphorus of germ rapidly decreased upon storage, particularly at high moisture contents.

The studies of Rich (1934), Sullivan, Near, and Foley (1936, 1936a), Bull (1937), Sullivan, Howe, and Schmalz (1936, 1937), Sullivan and Howe (1937), and Sullivan, Howe, Schmalz, and Astleford (1940) clearly showed that the phosphatides or other lipid materials were not responsible for the injurious effects of wheat germ or for the beneficial effects of oxidizing improvers. The harmful constituents were found to be soluble in water rather than in lipid solvents and ether-extracted flours will give a positive baking response to oxidizing agents. Sullivan and her co-workers identified the presence of glutathione in wheat germ, observed that it had a decidedly harmful effect upon flour baking properties, and presented evidence that its oxidation is in large measure responsible for the beneficial effect of heat treatment on wheat germ and germ-containing flour streams. These studies have directed attention to the importance of sulfhydryl compounds and oxidation-reduction phenomena in relation to flour improvement. The lipids do, however, appear to play a role in the changes in baking quality due to natural aging. It is well known that flour acidity increases on aging and Kozmin (1935), Barton-Wright (1938) and others have shown that the colloidal behavior of gluten is materially influenced by unsaturated fatty acids. In view of the independent discovery by Balls and Hale (1940) and Sullivan, Howe, Schmalz, and Astleford (1940) of a sulfur-containing constituent in flour lipid extracts, the lipids may eventually be found to play some part in oxidation-reduction phenomena during dough fermentation. Reference must also be made to the suggestion put forward by Bungenberg de Jong (1938) that gluten interacts with lecithin, thereby

making it possible for hydrophobic substances such as fats and sterols to be bound to the protein. The solvation of this complex is influenced by unsaturated fatty acids and the system is sensitive to oxidation-reduction processes.

Jørgensen (1935, 1935a, 1936, 1938), in experiments in which the increase in water-soluble nitrogen upon autolysis of flour suspensions was determined, reported that wheat flour contains powerful but latent proteolytic enzymes which are activated by glutathione and yeast water and inhibited by potassium bromate and iodate (but not by potassium chlorate) and ascorbic acid. Likewise, potassium bromate and iodate, but not potassium chlorate, also inhibited the protein-splitting action of papain, wheat germ, pineapple, liver, and flour proteases when acting on gelatin. Since potassium bromate and iodate are flour improvers, while potassium chlorate is not, Jørgensen proposed the theory that potassium bromate and analogous oxidizing agents exert their beneficial effects on baking quality by inhibiting more or less completely the flour proteinases, which are rendered active by the yeast and glutathione, thereby diminishing the breakdown of the gluten proteins during fermentation and improving the gas-retaining capacity of the doughs. Likewise the important studies of Balls and Hale (1935, 1936, 1936a, 1938) and of Hale (1939) on the proteinase enzymes of wheat bran and wheat flour led them to conclude that the inactivation of wheat proteinase by oxidizing improvers adequately explains their action as bread improvers when added to fermenting doughs.

The proteolytic theory of Jørgensen and of Balls and Hale was opposed by Read and Haas (1937, 1938, 1939), who repeated certain of Jørgensen's extraction studies and pointed out that the ratio of potassium bromate to flour employed by him greatly exceeded the amount used in baking practice. When used at commercial levels they secured no significant repression of proteinase activity; moreover they reported that the proteinase of malted wheat flour was not as readily inhibited by bromate as papain and bromelin. In answer to Read and Haas, Jørgensen (1939) pointed out that because of the wider flour-water ratio in the extraction tests, larger amounts of bromate would be required to produce effects corresponding to those in dough; moreover, in a fermenting dough, yeast activates the flour proteinases so that the amounts of bromate necessary to show a reduction in nitrogen solubility in extraction tests cannot be directly compared with the quantities effective in bread doughs. Further experiments were then reported by Jørgensen which indicated that, when due consideration is given to these points, dosages of bromate corresponding to those used in baking reduce the solubility of the

flour nitrogen. Flohil (1936) gave extensive evidence of the depressing action of flour improvers on proteolytic activity. He found that wheat germ was exceedingly rich in proteases and attributed its harmful effects on baking value to this fact, pointing out that, in general, the response to improvers increases with increasing germ content.

It must be clearly recognized, as has been pointed out by Sullivan, Howe, Schmalz, and Astleford (1940), that the proteinase theory is not based on direct evidence but rather on an interesting parallelism between the influence of certain reducing and oxidizing agents on the activity of flour proteases and the general effects of these substances on flour baking properties. Reducing agents such as cysteine and reduced glutathione stimulate flour protease activity and have a detrimental effect on gluten quality and flour baking properties, whereas oxidizing agents, such as nitrogen trichloride, persulfates, bromates, and iodates, have an inhibitory effect on flour protease activity and are flour improvers; on the other hand, potassium chlorate is essentially without influence on either protease activity or flour baking properties. Although many investigators have shown that the addition of proteases to flour results in excessive dough slackening and serious injury to baking properties, such experiments do not prove that proteolytic activity is, in itself, of any particular significance in relation to the baking behavior of normal flours. This theory has served a useful purpose in stimulating interest in the complicated problem of the mode of action of oxidizing and reducing agents in fermenting doughs but further progress demands that its limitations be generally recognized. The theory is not in accord with the fact that the lower the grade of flour milled from any particular wheat, the longer, in general, is the fermentation time required to produce an optimum loaf. Also, it fails to explain satisfactorily the harmful baking effects of excess bromate.

Ford and Maiden (1938) carried out farinograph studies on flour doughs to which glutathione and papain, respectively, had been added. Glutathione had a direct and immediate softening effect, whereas papain had, as well as an immediate action, a very marked delayed softening effect of the type one would expect as a result of increased protease activity. On the other hand the softening of the doughs containing glutathione did not increase with time to the extent that would be expected if it functioned mainly as an activator of flour proteases. In the light of these results, Ford and Maiden believe that the baking effects of glutathione are due primarily to a direct action on the flour proteins themselves and not to flour protease activation.

Upon testing doughs with the extensograph at varying intervals of time after molding, Munz and Brabender (1940) found that the extensibility (E) of the doughs decreased and the resistance to extension (F) increased with rest time. Corresponding bromated and nonbromated doughs gave similar curves immediately after mixing, but after a period of rest the tightening effect of the bromate became evidenced by a substantial increase in the F/E ratio. When potassium persulfate was used instead of bromate, a more immediate effect was registered. In the instance of flours which gave a strong positive response to bromate, the area under the extensogram curve was markedly increased. The extensibility of doughs containing papain and cysteine was greater and the resistance to extension less than for normal doughs tested at corresponding time intervals. The differences between these values for the normal and treated doughs increased with time, thus providing physical evidence in support of the inhibitory influence of bromate on the action of papain and similar proteolytic enzymes.

Baker and Mize (1937, 1939) have carried out tests on the effects of mixing doughs in vacuum and in the presence of such gases as air, oxygen, nitrogen, and hydrogen, with and without the addition of inorganic peroxides and potassium bromate. They found that doughs could be mixed for prolonged periods in vacuum or inert gases without deterioration. However, deterioration occurred when oxygen or inorganic oxidizing agents were added to the doughs. The action of bromate was found to be promoted by mechanical mixing. In the absence of both mechanical action and yeast fermentation, bromate had little effect.

Freilich and Frey (1939, 1939a, 1939b) found that potassium bromate produced effects in addition to and apparently different from direct inhibition of proteolytic activity. Excessive amounts of bromate produced great decreases in loaf volume, the "excess bromate effect" being greater for any given dosage level with longer fermentation time. They point out that if the effect of bromate were merely to inhibit protease activity, there should be no further effects when sufficient amounts of the oxidizing agent to accomplish this purpose have been added. It was found that the undesirable effects produced by excessive amounts of bromate, or by prolonged fermentation, could be eliminated by remixing the doughs after fermentation. These investigators also carried out numerous experiments in which the doughs were mixed in oxygen. Mixing in oxygen reduced the harmful effects of added pepsin, wheat germ, cysteine, and glutathione, and the production of amino nitrogen was markedly retarded.

Sullivan and her associates have made important contributions in this field. Recently Sullivan, Howe, Schmalz, and Astleford (1940) have reviewed the literature and presented additional studies on the fundamental nature of the action of oxidizing agents as flour improvers. They concluded that the starch, lipids, sugars, and diastatic enzymes of the wheat flour are of secondary importance and pointed out the insufficiency of the proteolytic theory to explain all the known facts. They believe that the detrimental effect of glutathione and other sulfhydryl compounds in relation to baking quality of flour is due mainly to their direct action on the colloid properties of the gluten and conclude that the effect of oxidizing and reducing agents on flour can be best explained, with the facts at present available, on the basis of their direct action on the sulfhydryl linkage of the flour proteins.

Freilich and Frey (1940), employing an iodine titration method developed by Freilich (1941), determined the reducing matter in low-grade, clear, straight-grade, and patent flours milled from both Texas and northwestern wheats. Protease activity was also determined by a formol titration procedure on doughs mixed from these flours after standing 24 hours at 86° F. No yeast was added in mixing the doughs since it was found that the amino nitrogen produced by proteolytic activity was utilized by the yeast. The activity of any wild yeasts which might have been present was inhibited by octyl alcohol which was shown to have no effect on protease activity. The results of these determinations were correlated with the results of baking tests conducted on doughs mixed in oxygen, and nonbromated and bromated doughs mixed in nitrogen, respectively. The protease activity and reducing-matter contents of the various flour grades were found to be positively related. Similarly protease activity and reducing-matter contents were each positively correlated with the increase in loaf volume due to oxidation. These workers suggest that these two factors may be of about equal importance as indicators of baking improvement resulting from the use of oxygen or oxidizing agents.

The effect of skim-milk solids on baking behavior is of interest in connection with the action of oxidizing agents. The experiments of Greenbank, Steinbarger, Deysher, and Holm (1927), Grewe and Holm (1928), Skovholt and Bailey (1931, 1932), Johnson and Ward (1936), and others, show quite definitely that the "baking quality" of milk may be improved by suitable heat treatment. Ofelt and Larmour (1940) have made the important observation that the addition of skim-milk solids not only increases the bromate requirement for optimum baking results but also has a buffering effect, increasing the tolerance towards excessive dosages. Treatment of two flours with the protease activator cysteine monochloride did not accentuate their

differential response to bromate, indicating that the protease content of the flours was not responsible for the differences. The addition of cysteine produced a very marked and almost instantaneous effect on the physical properties of the doughs which, according to these authors, was too rapid to be attributed to enzyme action and appeared to have the characteristics of a colloidal effect. Eisenberg (1940) has confirmed the buffering effect of milk solids on bromate.

Investigations of the thiol groups of proteins by Hopkins (1930, 1930a), Mirsky and Anson (1935, 1936), Greenstein (1938, 1939, 1939a) and others, reveal that the heat denaturation of proteins is accompanied by an increase in sulfhydryl groups. Gould and Sommer (1939, 1939a) have shown that normal milk gives a negative nitroprusside test, but when it is heated to 90° C a faint positive reaction is obtained. Moreover, a distinct increase in the intensity of the color reaction upon treatment with cyanide was obtained with milks heated to temperatures of 76°–80° C, indicating greater ease of reduction of the sulfur linkages as a result of heat treatment. The development of the cooked flavor upon heating was associated with the formation and liberation of hydrogen sulfide, and a lowering of the oxidation-reduction potential. These observations suggest that the improvements in the "baking quality" of milk resulting from heat treatment, and the effect of milk on bromate requirement and tolerance, may be related to changes in the sulfur linkages.

Recently Ziegler (1940, 1940a, 1940b, 1941) has published a series of papers relative to glutathione oxidation and the effects of the reduced and oxidized forms on dough properties as measured by Chopin extensimeter and farinograph tests.

In aqueous solution at temperatures below 40°C, reduced glutathione (GSH) was slowly oxidized by potassium bromate, but very rapidly oxidized by iodine at pH 4.5–5.6. Farinograph studies indicated that the oxidation of GSH by bromate was not catalyzed by yeast or the constituents of a nonfermenting dough. While GSH additions to flour doughs yielded soft sticky doughs and lowered the loaf volume, very small amounts of GSH had a beneficial effect. Sodium chloride in proportions used in baking partially protected the gluten against the harmful action of GSH. Oxidized glutathione was apparently reduced again in nonfermenting doughs, probably due to the accumulation of reducing sugars. Oxidation of aqueous GSH solutions with ammonium persulfate required more than thirty times greater concentration than of potassium bromate while only about three times the concentration is needed for flour improvement, indicating that the improving action of persulfates may be due to factors other than GSH oxidation. A study was also made of the effect on

gluten swelling properties and flour baking behavior of various substances known to be involved in, or influencing, respiratory rate. Succinic acid, fumaric acid, and adrenaline, which increased respiration, improved baking quality, although only adrenaline in the presence of a trace of copper actually inhibited proteolysis.

Iodine titration and gluten swelling studies showed that the flour-improving effect of bromate, iodate, persulfate, and dehydroascorbic acid could at least partly be explained by their ability to oxidize glutathione. These substances also inhibited the breakdown of gluten by commercial papain, but Ziegler pointed out that as the papain preparation may have contained an activator, these results cannot be interpreted as proving that the oxidizing agents act directly on the enzyme itself. Ziegler interpreted his studies as being in line with the Jørgensen theory.

Sullivan, Howe, Schmalz, and Astleford (1940) have pointed out that the amount of bromate required to produce optimum response is greater with brake than with normal doughs and have confirmed the observations of Freilich and Frey (1939a) that overtreated doughs can be brought back to normal on remixing. Neither this excess effect of oxidizing agents nor its amelioration by purely physical means can be satisfactorily explained by the theories of Jørgensen and of Balls and Hale. Sullivan and coworkers also point out that different flour improvers produce somewhat different effects on flour doughs; for example, iodate exhibits a beneficial action on dough much earlier in the fermentation than does bromate, which is in line with the observation that iodate does not require as low an acidity to function as an oxidizing agent.

This general review of the literature indicates that the problems involved in the mechanism of the action of oxidizing agents in fermenting doughs are by no means simple. It is clear, however, that their baking effects are due primarily to some action involving the gluten proteins, but on purely theoretical grounds there are several ways in which modifications in the properties of the flour proteins could be brought about. These include a direct action on the sulfur linkages of the gluten and indirect effects which might be brought about by a number of possible ways. The colloidal character of the gluten might be influenced indirectly with or without oxidation of the gluten itself by an intermediate substance which has been oxidized by the flour improver. On the other hand, the oxidizing improvers could function by decreasing proteolytic activity either by direct oxidation of the proteinase enzyme itself or by conversion of an activator, through oxidation, into an inactive form.

The question arises as to how many of these possible mechanisms are actually functioning when oxidizing agents are added to fermenting doughs, and if several are operative, which of these are of primary importance. The literature at present available clearly indicates that more than one mechanism is involved. The inhibition of protease activity by oxidizing improvers has been well established, and flours of high protease activity and reducing-matter content give marked baking responses to oxidizing improvers. However, the very rapid effect of glutathione, wheat germ, and other reducing substances on dough properties, the lessening of their harmful effects upon extension of the fermentation time, coupled with the "excess bromate effect" and its amelioration by purely physical means (remixing after fermentation), strongly indicate that proteinase activity is not of primary importance in relation to the baking effects of oxidizing agents. Moreover, if active proteolysis taking place during fermentation has a marked degrading effect on the physico-chemical properties of the flour proteins one would anticipate that the low-protein, weak flours would show the greatest response to oxidizing improvers. The fact that the high-protein flours give the greatest response can apparently be interpreted only in terms of the proteolytic theory by assuming that such flours have a higher protease and/or reducing-matter content.

On the basis of our present knowledge, two other possible mechanisms seem to be much more attractive than the proteinase theory: (1) the oxidation of glutathione and other reducing substances in the dough which exert a direct effect on the colloidal properties of the flour proteins rather than an indirect effect through the medium of proteinase activation, and (2) the direct oxidation of the sulfhydryl linkages of the flour proteins. Sullivan, Howe, Schmalz, and Astleford (1940) have proposed two explanations of improver action based on these possibilities. They favor the theory that dough fermentation, physical manipulation of the doughs, and oxidizing and reducing agents bring about changes in the sulfur linkages of the gluten proteins. In accord with observations that protein denaturation results in marked changes in the physical properties of various proteins and is accompanied by an increase in RSH groups, it is suggested that during fermentation, alteration or cleavage of R-S-S-R linkages may take place, with the production of, for example, RSH groups, some of which may be oxidized by air or oxidizing improvers, the rate of oxidation being dependent upon their degree of dissociation and the oxidation potential of the fermentation. This theory is particularly attractive since it provides a common basis for explaining flour improvement by physical and chemical means. In the light of the above theory it would be of interest to follow the changes in the reducing-

matter content of fermenting doughs and, in particular, to determine the influence of potassium bromate on the reducing matter levels. In the studies of Freilich and Frey (1940), proteolytic activity was determined in nonyeasted doughs (with the addition of sufficient octyl alcohol to prevent any activity of wild yeasts) after a fixed time, and reducing matter was determined in the flour. The objectives of the present study were to extend their work by determining the amino nitrogen and reducing-matter content of actual fermenting doughs baked with and without bromate for varying fermentation times and to ascertain whether any relation exists between the extent of proteolysis and the reducing matter content of the doughs at the end of the proof period, and (1) the volume of the baked loaf and (2) the loaf volume response to bromate.

Experimental

The experimental material consisted of three grades of flour: short patent, fancy clear, and low grade from the same commercial mill mix of hard red spring wheat. The protein and ash values of the flours, which were all untreated, are given in Table I.

TABLE I

PROTEIN AND ASH CONTENT OF FLOURS EMPLOYED

Flour grade	Crude protein [1]	Ash [1]
	%	%
Short patent	12.5	0.42
Fancy clear	15.8	0.72
Low grade	16.3	1.02

Each flour was baked with additions of nil, 0.001, 0.002, 0.004, and 0.006% potassium bromate with fermentation times of 1.5, 3.0, and 4.5 hours respectively. Reducing matter and amino nitrogen content were determined after mixing and again at the end of the proof period on doughs made without and with 0.004% potassium bromate for each of the above fermentation times. These determinations were made on each of the respective doughs with and without the addition of octyl alcohol.

Experimental baking methods: The basic formula (to which the required additions of potassium bromate were made) was as follows: flour (15% moisture basis) 100 g, yeast (Fleischmann's) 3 g, sucrose 5 g, sodium chloride 1 g, and sufficient distilled water to yield a dough of the desired consistency. Three hundred grams of flour was mixed with the proportional quantities of the other dough ingredients in a

[1] Expressed on 13.5% moisture basis.

Hobart-Swanson mixer for two minutes and scaled into three portions of 150 g each for the three different fermentation times. The doughs were fermented at 30°C with the following schedule for punching and molding:

Total fermentation time	Time after mixing			
	First punch	Second punch	Third punch	Molding
hrs	*min*	*min*	*min*	*min*
1.5	65	nil	nil	90
3.0	105	155	nil	180
4.5	120	195	245	270

All doughs were proofed 55 minutes at 30°C and baked for 25 minutes at approximately 230°C. The baking tests were carried out in duplicate, the replicate loaves being baked on different days. Loaf volume was measured approximately two hours after removing from the oven and the loaves were scored the following day.

Determination of amino nitrogen and reducing matter in doughs: Since soluble nitrogen compounds are utilized by actively fermenting yeast, it is necessary, as pointed out by Freilich and Frey (1940), to inhibit yeast fermentation or to make the tests on doughs fermented to the same extent. These workers have shown that yeast activity can be inhibited by a suitable concentration of octyl alcohol—a reagent which was found to be without influence on proteolytic activity. Since in our studies it was desired also to determine reducing matter in the doughs at the end of the proof period, a preliminary experiment was carried out to ascertain the effect of yeast and octyl alcohol on the level of reducing matter. Doughs were prepared from the low-grade flour by the formula employed for the experimental baking tests as follows: (1) basic dough, (2) basic dough + octyl alcohol, (3) basic dough without yeast, (4) basic dough without yeast + octyl alcohol. Octyl alcohol, where required, was used at the rate of 0.3 ml per 100 g of flour, the concentration which was found by Freilich and Frey (1940) to be effective in inhibiting yeast fermentation.

Determinations of amino nitrogen and reducing matter were made for each of the above doughs 1.5, 3.0, and 4.5 hours, respectively, after mixing. For each of these times, doughs were prepared with 200 g of flour and the other ingredients (with or without yeast and/or octyl alcohol as required for the different series outlined above) as described for the experimental baking tests. Two 150-g doughs were scaled, punched, fermented, and proofed in the manner employed in carrying out the baking tests. After proofing, the duplicate doughs were placed together in a Waring Blendor, the glass container of which

was modified in a manner similar to that described by Freilich and Frey (1940) by reducing its capacity and grinding the top edges and fitting thereto a glass plate through which passed two glass tubes equipped with stopcocks.

Two grams of sodium chloride and 500 ml of distilled water were added, the glass cover placed in position, nitrogen gas passed in, and the dough disintegrated by operating the Blendor for 2.5 minutes. This treatment resulted in a uniform suspension. A portion of the suspension was centrifuged and the amino nitrogen determined in 10 ml of the supernatant liquid by a modification of the Sorenson formol titration procedure suggested by Samuel (1934) using $N/14$ NaOH solution. This procedure, fully described in *Cereal Laboratory Methods* (4th ed., 1941), involves the use of phenol red indicator and titration to pH 8.0 as determined by matching against a buffer solution of this pH in a comparator block. By centrifuging at high speed for 10 minutes to secure a reasonably clear extract and titrating immediately after addition of the indicator, very satisfactory end points were obtained. The results were calculated in terms of milligrams of amino nitrogen in a dough weight equivalent to 100 g of flour (15.0% moisture basis).

For the determination of reducing matter, the method recently described by Freilich (1941) was employed. This is based upon oxidation of an extract of the flour with iodine at approximately pH 3.0 from which the proteins have been precipitated by sulfosalicylic acid and sodium chloride. Half of the suspension prepared from the dough was allowed to stand in a stoppered bottle for 45 minutes, and 10 ml of 60% sulfosalicylic acid solution added with slight shaking. After 15 minutes the mixture was centrifuged in a glass-stoppered bottle and the extract filtered through folded paper. Immediately after filtering 5 ml of $0.005N$ iodine solution was added to 50 ml of the clarified extract and the solution back titrated with $0.005N$ sodium thiosulfate solution using 2 ml of 1% starch solution as indicator. The results were expressed as ml $0.001N$ iodine consumed by a quantity of the dough extract equivalent to 100 g of flour (15.0% moisture basis). Experiments in which the use of nitrogen gas was eliminated and the titrations made at different times after filtration of the centrifuged extract confirmed the observations of Freilich (1941) that there is a gradual loss of reducing matter upon exposure to air. Accordingly, nitrogen gas was always employed and the same time schedule was adhered to from sample to sample in order to secure as comparable results as possible.

The values recorded in Table II confirm the findings of Freilich and Frey (1940) regarding the necessity of inhibiting fermentation if

TABLE II

EFFECT OF YEAST AND FERMENTATION (OR REST) TIME ON AMINO NITROGEN AND REDUCING MATTER CONTENT OF DOUGHS MADE WITH LOW GRADE FLOUR

Fermentation[1] (or rest) time	Basic dough		Basic dough without yeast	
	Without octyl alcohol	With octyl alcohol	Without octyl alcohol	With octyl alcohol
	AMINO N PER DOUGH WEIGHT EQUIVALENT TO 100 G FLOUR			
hrs	*mg*	*mg*	*mg*	*mg*
1.5	22.1	28.2	22.1	21.3
3.0	7.1	31.7	24.4	22.3
4.5	6.1	33.5	25.6	22.8
	REDUCING MATTER (ML) 0.01*N* IODINE PER DOUGH WEIGHT EQUIVALENT TO 100 G FLOUR			
hrs	*ml*	*ml*	*ml*	*ml*
1.5	10.3	14.0	8.6	8.5
3.0	11.0	13.9	9.9	9.2
4.5	9.5	14.4	10.5	11.1

[1] Proofing time 55 minutes is not included in the times recorded.

the amino nitrogen content of the doughs after varying rest times is to be taken as a measure of proteolytic activity. In general, the presence of yeast increased the reducing matter content of the dough, particularly when octyl alcohol was added. In view of the results of these preliminary experiments it was deemed advisable to determine amino nitrogen and reducing matter content of basic and bromated yeast doughs made both with and without the addition of octyl alcohol. The effect of bromating was ascertained only for the 0.004% dosage. The determinations were made by the methods already described on the doughs immediately after mixing and also after 1.5, 3.0, and 4.5 hours of fermentation.

Results

The mean loaf volumes for each flour, bromate dosage, and fermentation time are recorded in Table III and graphically depicted by the histograms given in Figure 1. The data for the amino nitrogen and reducing matter content of the basic and bromated doughs (4 mg level only) are given in Table IV and Figure 2.

The baking results confirm previous observations (Geddes and Larmour, 1933) regarding the greater response and greater tolerance of fancy clear and low-grade flours, to bromate as compared with the patent flour. At 1.5 hours of fermentation all three flours gave a

TABLE III

LOAF VOLUME DATA FOR VARIOUS BROMATE DOSAGES AND FERMENTATION TIMES

Bromate per 100 g flour	Mean loaf volumes with fermentation time in hours:		
	1.5 hrs	3.0 hrs	4.5 hrs
mg	*cc*	*cc*	*cc*
	PATENT FLOUR		
0	563	595	583
1	668	585	558
2	670	580	485
4	695	485	360
6	685	425	350
	FANCY CLEAR FLOUR		
0	590	615	565
1	668	793	755
2	708	915	685
4	963	663	470
6	980	668	413
	LOW-GRADE FLOUR		
0	555	545	538
1	590	643	670
2	615	758	618
4	718	600	405
6	810	510	380

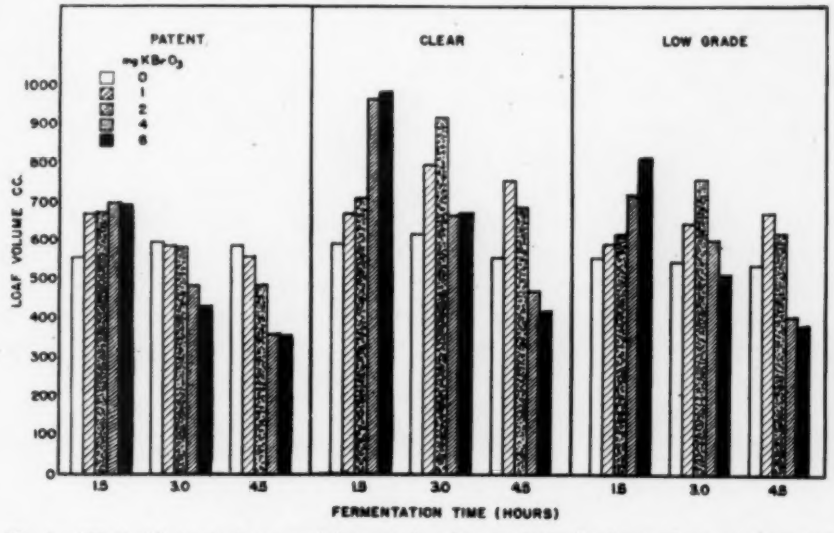

Fig. 1. Effect of bromate dosage and fermentation time on the loaf volumes of patent, clear, and low-grade flours.

positive response to bromate, the response increasing with the bromate dosage, except in the case of the patent flour which gave an optimum response at the 4-mg level. At 3.0 and 4.5 hours of fermentation time the positive responses in loaf volume were limited to the lower bro-

TABLE IV

AMINO NITROGEN AND REDUCING MATTER CONTENT OF DOUGHS

Treatment per 100 g flour		Amino N per 100 g flour—fermentation (or rest) time in hours:[1]				Reducing matter 0.01N iodine per 100 g flour—fermentation (or rest) time in hours:[1]			
Bromate	Octyl alcohol	0	1.5	3.0	4.5	0	1.5	3.0	4.5
mg	*ml*	*mg*	*mg*	*mg*	*mg*	*ml*	*ml*	*ml*	*ml*
				PATENT FLOUR					
0	0	8.6	1.5	0.3	0.3	2.3	5.4	6.7	5.7
4	0	7.6	0.0	0.0	1.3	.6	3.6	4.3	4.4
0	3	18.0	19.5	22.1	22.8	4.2	5.1	5.7	5.4
4	3	18.5	20.8	21.3	23.3	3.4	2.0	1.9	2.7
				FANCY CLEAR FLOUR					
0	0	13.9	6.1	2.8	2.3	3.5	6.1	6.2	7.0
4	0	10.4	1.8	1.5	3.0	3.1	5.5	5.1	4.5
0	3	22.0	25.6	29.2	29.7	6.6	8.4	9.8	10.3
4	3	22.3	25.4	27.1	28.4	5.7	6.8	6.6	6.5
				LOW-GRADE FLOUR					
0	0	15.5	22.1	8.6	6.1	5.9	10.3	11.0	9.5
4	0	9.4[2]	3.0	3.0	8.1	5.3	6.8	7.3	3.4
0	3	24.9	28.2	31.7	33.5	9.8	14.0	13.9	14.4
4	3	23.1	27.9	29.4	32.2	8.1	8.8	9.8	10.1

[1] Proof period of 55 minutes not included in times given.
[2] Single determination only.

mate dosages in both low-grade and fancy clear flours while all bromate treatments gave negative responses with patent flour; thus the greater the bromate dosage, the shorter the fermentation time required to give optimum baking results. The handling properties of the doughs changed materially as the fermentation time was extended; those made with high bromate dosages gradually became "tough" and "solid."

The amino nitrogen content of the doughs at mixing time (zero hours of fermentation) increased with decreasing flour grade. In all three grades of flour both the basic and bromated doughs showed a gradual increase in amino nitrogen content with increasing "rest time" when yeast activity was inhibited by addition of octyl alcohol. In the presence of yeast fermentation, there was, in general, a pronounced drop in amino nitrogen with increasing fermentation time as a result of the utilization of amino nitrogen by the active yeast cells.

There was always a somewhat lower content of amino nitrogen in the bromated as compared with the corresponding nonbromated doughs

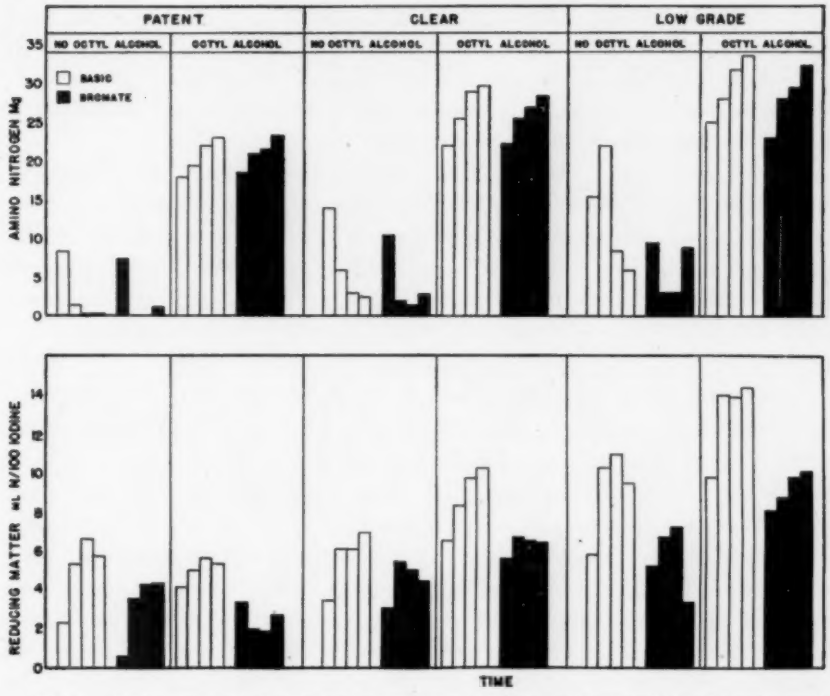

Fig. 2. Amino nitrogen and reducing matter content of basic and bromated (4 mg) doughs made with and without the addition of octyl alcohol after various resting or fermentation times. From left to right, the bars in each section represent the values determined on doughs after 0, 1.5, 3.0, and 4.5 hours, respectively.

containing active yeast but in doughs containing octyl alcohol the apparent depressing effect of bromate was very slight.

The reducing-matter content increased with decreasing flour grade and with the exception of the patent flour the amount of reducing matter was uniformly higher whenever yeast activity was inhibited by octyl alcohol. With a few exceptions the reducing-matter content increased with fermentation. In every case the reducing matter of corresponding doughs was very much less whenever bromate was added, the decrease due to bromate being greater the larger the quantity of reducing matter present.

Discussion

In comparing these results with those obtained by Freilich and Frey (1940), it must be emphasized that they determined reducing matter in flours and not in doughs after varying fermentation times; moreover, they determined amino nitrogen in doughs allowed to ferment or to rest (octyl-alcohol-treated doughs) for 24 hours. In the present study, however, both reducing matter and amino nitrogen

were determined on doughs at mixing and at the end of the proof period after 1.5, 3.0, and 4.5 hours of fermentation or resting time. The data for amino nitrogen and reducing matter content are of particular interest because they represent the levels at the end of the proof period under actual baking conditions.

The amino nitrogen values fully confirm the observations of Jørgensen (1935, 1935a, 1936) that bromate exerts a greater inhibitory effect on proteolytic activity when active yeast is present, as judged by the differences in amino nitrogen content at corresponding fermentation times. In doughs containing octyl alcohol, which inhibits fermentation, the results are not entirely consistent but in general indicate a slight depressing effect due to bromate.

Unfortunately the utilization of amino nitrogen by actively fermenting yeast renders it impossible to use the amino nitrogen values after varying fermentation times as a measure of the proteolysis which has occurred. In the instance of the doughs treated with octyl alcohol, amino nitrogen increased with time, and because of the apparent stimulating effect of the yeast it may be assumed that greater proteolysis occurred in the fermenting doughs. Accordingly the relative increase in amino nitrogen content of the basic and bromated octyl-alcohol-treated doughs from the time of mixing may be regarded as minimum values for proteolytic activity. These are recorded in Table V along with the basic and bromate volumes and reducing matter contents for the fermenting doughs. The differences between the corresponding basic and bromate data for the three variables are also recorded.

It is of interest to note that patent flour which has the lowest proteolytic activity and reducing-matter content has the lowest bromate response of the three flours. However, considering the data as a whole, one could not predict the behavior of the flours toward bromating or toward increased fermentation from a knowledge of the amount of proteolysis or reducing-matter content of the basic doughs at a given time after mixing. Bromate and fermentation have a supplementary effect upon loaf volumes, resulting in overdevelopment of the doughs when heavy bromate treatment is combined with extended fermentation, yet bromate reduces the extent of proteolysis and decreases the reducing-matter content, whereas lengthening of the fermentation results in increased proteolysis and also in general in an increase in reducing-matter content. The percentage depression in reducing matter due to bromate is much greater than the indicated percentage depression in proteolysis but it must be remembered that the amino nitrogen values do not represent the conditions in actual fermenting doughs.

TABLE V

EFFECT OF BROMATE TREATMENT (0.004%) AND FERMENTATION ON LOAF VOLUMES AND REDUCING MATTER CONTENT; EFFECT OF BROMATE AND RESTING TIME ON PROTEOLYTIC ACTIVITY

Fermentation time	Loaf volume			Proteolysis[1]—amino N per 100 g flour			Reducing matter—0.01N I_2 per 100 g flour		
	Bromate	Basic	Response	Bromate	Basic	Response	Bromate	Basic	Response
hrs	*cc*	*cc*	*cc*	*mg*	*mg*	*mg*	*ml*	*ml*	*ml*
				PATENT FLOUR					
1.5	695	563	+132	1.5	2.3	−0.8	3.6	5.4	−1.8
3.0	485	595	−110	4.1	2.8	+1.3	4.3	6.7	−2.4
4.5	360	583	−223	4.8	4.8	0.0	4.4	5.7	−1.3
				FANCY CLEAR FLOUR					
1.5	963	590	+373	3.1	3.6	−0.5	5.5	6.1	−0.6
3.0	663	615	+ 48	4.8	7.2	−2.4	5.1	6.2	−1.1
4.5	470	565	− 95	6.1	7.7	−1.6	4.5	7.0	−2.5
				LOW-GRADE FLOUR					
1.5	718	555	+163	4.8	3.3	+1.5	6.8	10.3	−3.5
3.0	600	545	+ 55	6.3	6.8	−0.5	7.3	11.0	−3.7
4.5	405	538	−133	9.1	8.6	−0.5	3.4	9.5	−6.5

[1] The values were obtained by subtracting the amino nitrogen levels in the octyl alcohol treated at mixing times from those at specified stages in the fermentation.

Caution must be observed in making broad generalizations from biochemical studies of this nature, especially as there is some doubt as to the adequacy of the methods. Several workers have pointed out that significant changes in the colloidal properties of the flour proteins, as reflected in dough behavior, may take place with little measurable increase in amino nitrogen. Moreover this determination measures peptidase (if present) as well as proteinase activity. In evolving a workable theory of bromate action it would be desirable to determine whether there is a direct proportionate relationship between proteinase-activity depression and reducing-matter decrease by bromating. This requires a method which is specific for proteinases and applicable to fermenting doughs.

The method for reducing matter also leaves much to be desired in view of the difficulty of preventing oxidation during the determination. Information is needed as to precisely what substances in dough are contributing to the values obtained. There is also the question as to whether the determination of reducing matter at pH 3.0 (approximately) yields results which would parallel those which would be

obtained at the higher pH values of fermenting doughs, if consistent results could be secured within such pH ranges. Accepting the results at their face value, they indicate a definite increase in RSH compounds with time in the instance of nonfermenting doughs, made by the basic formula. In general there is also an increase in RSH in the fermenting doughs made by this formula up to three hours of fermentation. It must be noted, however, that the nonfermenting doughs were rendered nonfermenting by octyl alcohol, which may have modified the permeability of the yeast cells and permitted the diffusion of yeast glutathione into the dough solution. It does not appear that reducing sugars, which would accumulate in the nonfermenting doughs, would contribute to the iodine titration at the low pH employed.

In view of the limitations discussed above, the authors prefer not to draw conclusions as to the mechanism of bromate action in dough fermentation. The complete elucidation of this problem must await the development of more satisfactory techniques.

Summary

Baking tests on short patent, fancy clear, and low-grade hard red spring wheat flours made with additions of nil, 0.001%, 0.002%, 0.004%, and 0.006% of potassium bromate at fermentation times of 1.5, 3.0, and 4.5 hours showed the loaf-volume response and tolerance to bromate to be greatest for the fancy clear and low-grade flours. For each flour the bromate response decreased with extension of the fermentation time.

Amino nitrogen and reducing-matter content of the doughs at mixing time increased with decreasing flour grade.

When yeast fermentation was inhibited by octyl alcohol, proteolytic activity of the doughs, as measured by the increase in amino nitrogen from mixing to the end of the proof, increased with fermentation time and was only slightly depressed by bromate. Proteolytic activity increased with decreasing grade. In actively fermenting doughs amino nitrogen was utilized by the yeast, but bromate considerably depressed proteolytic activity as indicated by the lower amino nitrogen levels at corresponding fermentation times.

Reducing-matter content of nonfermenting doughs (octyl-alcohol-treated), increased more with time than that of fermenting doughs. Bromate had a marked depressing effect which was more pronounced the longer the fermentation.

In view of the limitations of the analytical procedures employed, no conclusions are drawn as to the mechanism of bromate action in dough fermentation.

Literature Cited

Bailey, C. H.
1925 The chemistry of wheat flour. Chemical Catalog Company, N. Y.

Baker, J. C., and Mize, M. D.
1937 Mixing doughs in vacuum and in the presence of various gases. Cereal Chem. **14**: 721–734.
1939 The relation of mechanical stirring to sponge doughs. Cereal Chem. **16**: 294–295.

Balls, A. K., and Hale, W. S.
1935 Proteolysis in flours. J. Assoc. Off. Agr. Chem. **18**: 135–140.
1936 Proteolytic enzymes of flour. Cereal Chem. **13**: 54–60.
1936a Further studies on the activity of proteinase in flour. Cereal Chem. **13**: 656–664.
1938 The preparation and properties of wheat proteinase. Cereal Chem. **15**: 622–628.
1940 A sulphur-bearing constituent of the petroleum ether extract of wheat flour (preliminary report). Cereal Chem. **17**: 243–245.

Barton-Wright, E. C.
1938 Studies on the storage of wheaten flour. III. Changes in the flora and the fats and the influence of these changes on gluten character. Cereal Chem. **15**: 521–541.

Bull, Joan E.
1937 An investigation into the cause of the degrading effect of wheat germ on the baking quality of flour. Cereal Chem. **14**: 244–256.

Bungenberg de Jong, H. L.
1938 Proteins in the flour industry. Symposium on Proteins. Amsterdam.

Eisenberg, S.
1940 Evidence in support of a recent paper concerning the effect of milk on the bromate requirements of flours. Cereal Chem. **17**: 476–479.

Flohil, J. T.
1936 The effect of chemical flour improvers on proteolytic action in relation to the gas-retaining capacity of fermenting doughs. Cereal Chem. **13**: 675–689.

Ford, W. P., and Maiden, A. M.
1938 Effects in dough of glutathione and papain. J. Soc. Chem. Ind. (transactions) **57**: 278–281.

Freilich, J.
1941 A method for the determination of reducing matter in flour. Cereal Chem. **18**: 129–137.

Freilich, J., and Frey, C. N.
1939 Dough oxidation and mixing studies. I. The action of potassium bromate in dough. Cereal Chem. **16**: 485–494.
1939a Dough oxidation and mixing studies. II. Effects of remixing after fermentation. Cereal Chem. **16**: 495–502.
1939b Dough oxidation and mixing studies. III. The effects of proteases and reducing substances on dough when mixed in oxygen. Cereal Chem. **16**: 503–512.
1940 Dough oxidation and mixing studies. V. Correlation between reducing matter, protease activity and oxidizing effects in dough. Paper presented at A. A. C. C. meeting, New York, 1940 (manuscript seen).
1941 Dough oxidation and mixing studies. IV. Effects of oxygen and potassium bromate in sponge doughs. Cereal Chem. **18**: 78–86.

Geddes, W. F.
1930 The influence of germ constituents on baking quality and their relation to improvement in flour induced by heat and chemical improvers. Can. J. Research **2**: 195–213.

Geddes, W. F., and Larmour, R. K.
1933 Some aspects of the bromate baking test. Cereal Chem. **10**: 30–69.

Gould, I. A., Jr., and Sommer, H. H.
1939 Effect of heat on milk with especial reference to the cooked flavor. Mich. Agr. Exp. Sta. Tech. Bul. 164.
1939a Cooked and oxidized flavors of milk as affected by ferrous iron. J. Dairy Sci. **22**: 1017–1023.

Greenbank, G. R., Steinbarger, M. C., Deysher, E. F., and Holm, G. E.
1927 Effect of heat treatment of skim milk upon the baking qualities of the evaporated and dried products. J. Dairy Sci. **10**: 335–342.

Greenstein, J. P.
1938 Sulfhydryl groups in proteins. I. Egg albumin in solutions of urea, guanidine, and their derivatives. J. Biol. Chem. **125**: 501–513.
1939 Sulfhydryl groups in proteins. II. Edestin, excelsin, and globulin in solutions of guanidine hydrochloride, urea and their derivatives. J. Biol. Chem. **128**: 233–240.
1939a Sulfhydryl groups in proteins. III. The effect on egg albumin of various salts of guanidine. J. Biol. Chem. **130**: 519–526.

Grewe, Emily, and Holm, G. E.
1928 Effect of variation in the method of manufacture on the baking quality of dry skim milk. Cereal Chem. **5**: 461–469.

Hale, W. S.
1939 The proteinase in wheat flour. Cereal Chem. **16**: 695–702.

Hopkins, F. G.
1930 Denaturation of proteins by urea and related substances. Nature **126**: 328–330.
1930a Denaturation of proteins by urea and related substances. Nature **126**: 383–384.

Johnson, A. H., and Ward, F. C.
1936 The relation between the viscosity of non-sweetened condensed skim-milk and baking quality. Cereal Chem. **13**: 550–559.

Jørgensen, Holger
1935 Ein Beitrag zur Beleuchtung der hemmenden Wirkung von Oxydationsmitteln auf proteolytische Enzymtätigkeit: Über die Natureder Einwirkung von Kaliumbromat und analogen Stoffen auf die Backfähigkeit des Weizenmehles. I. Biochem. Z. **280**: 1–37 (also published in abbreviated form in Muhlenlab. **5**: 113–126).
1935a Über die Natur der Einwirkung von Kaliumbromat und analogen Stoffen auf die Backfähigkeit des Weizenmehles. II. Biochem. Z. **283**: 134–145.
1936 On the existence of powerful but latent proteolytic enzymes in wheat flour. Cereal Chem. **13**: 346–355.
1938 Entwicklung der Theorie der Bromatwirkung, 1935–1938. Muhlenlab. **8**: 201–208.
1939 Further investigations into the nature of the action of bromates and ascorbic acid on the baking strength of wheat flour. Cereal Chem. **16**: 51–60.

Kozmin, N. P.
1935 The aging of wheat flour and the nature of the process. Cereal Chem. **12**: 165–171.

Mirsky, A. E., and Anson, M. L.
1935 Sulfhydryl and disulfide groups of proteins. I. Methods of estimation. J. Gen. Physiol. **18**: 307–323.
1936 Sulfhydryl and disulfide groups of proteins. II. The relation of number of SH and S-S groups and quantity of insoluble protein in denaturation and in reversal denaturation. J. Gen. Physiol. **19**: 427–438.

Munz, Emil, and Brabender, C. W.
1940 Extensograms as a basis of predicting baking quality and reaction to oxidizing agents. Cereal Chem. **17**: 313–332.

Ofelt, C. W., and Larmour, R. K.
1940 The effect of milk on the bromate requirements of flour. Cereal Chem. **17**: 1–18.

Read, J. W., and Haas, L. W.
1937 Baking quality of flour as affected by certain enzyme actions. III. Purified amylase and the relative proteolytic activity of amylolytic agents. Cereal Chem. **14**: 58–73.
1937a Studies on the baking quality of flour as affected by certain enzyme actions. IV. Potassium bromate and enzyme activity. Cereal Chem. **14**: 752–766.
1938 Studies on the baking quality of flour as affected by certain enzyme actions. V. Further studies concerning potassium bromate and enzyme activity. Cereal Chem. **15**: 59–68.

1939 Studies on the baking quality of flour as affected by certain enzyme actions. VI. Further studies relating to the activation and inhibition of flour proteinase. Cereal Chem. **16**: 60–70.

Rich, C. E.
1934 The chemical and physico-chemical changes induced in wheat flour by artificial maturing agents. Cereal Chem. **11**: 201–227.

Samuel, L. W.
1934 The determination of amino-acids in wheat flour. Biochem. J. **28**: 273.

Skovholt, O., and Bailey, C. H.
1931 Relation of quality in dry skimmilk to baking strength. Cereal Chem. **8**: 374–380.
1932 The effect of temperature and of the inclusion of dry skimmilk upon the properties of doughs as measured with the farinograph. Cereal Chem. **9**: 523–530.

Sullivan, B., and Howe, Marjorie
1937 The isolation of glutathione from wheat germ. J. Am. Chem. Soc. **59**: 2742.

Sullivan, B., Howe, Marjorie, and Schmalz, F. D.
1936 On the presence of glutathione in wheat germ. Cereal Chem. **13**: 665–669.
1937 An explanation of the effect of heat treatment on wheat germ. Cereal Chem. **14**: 489–495.

Sullivan, B., Howe, Marjorie, Schmalz, F. D., and Astleford, G. R.
1940 The action of oxidizing and reducing agents on flour. Cereal Chem. **17**: 507–528.

Sullivan, B., and Near, Cleo
1933 Change of crude lipids of wheat on storage. Ind. Eng. Chem. **25**: 100–103.

Sullivan, B., Near, Cleo, and Foley, G. H.
1936 The role of lipids in relation to flour quality. Cereal Chem. **13**: 318–331.
1936a The harmful effect of wheat germ on the baking quality of flour and the constituents responsible for this effect. Cereal Chem. **13**: 453–462.

Working, E. B.
1924 Lipoids, a factor influencing gluten quality. Cereal Chem. **1**: 153–158.
1928 The action of phosphatides in bread dough. Cereal Chem. **5**: 223–234.
1928a Some oxidizing effects of flour bleaching. Cereal Chem. **5**: 431–435.

Ziegler, E.
1940 Dough improvement studies. I. Oxidation of glutathione by potassium bromate. Cereal Chem. **17**: 460–468.
1940a Dough improvement studies. II. The effect of adding oxidized glutathione to wheat flour doughs. Cereal Chem. **17**: 551–555.
1940b Dough improvement studies. III. Further studies on the oxidation of glutathione by bromate, chlorate, and persulphate. Cereal Chem. **17**: 556–564.
1941 Dough improvement studies. IV. Effect of some factors involved in respiration on the baking quality of wheat flour. Cereal Chem. **18**: 167–185.

BOOK REVIEW

Advances in Colloid Science, Vol. I. By Elmer O. Kraemer, Editor, in collaboration with Floyd Bartell and S. S. Kistler. 434 pages. Interscience Publishers, Inc., 215 Fourth Avenue, New York. Price $5.50.

This volume is the first of a series intended to provide organized and comprehensive surveys of recent discoveries in colloid science. The contributions are not merely reviews or compilations; the experimental and theoretical aspects of each development under consideration are presented by authors who by their own backgrounds are able critically to evaluate the existing data and techniques and to present the material in a uniform fashion.

The first volume includes a wide range of subjects, all of front-line importance and written for those directly engaged in the respective fields as well as for others whose interests may extend into these fields. For those interested in molecular and micellar structure, size, and shape, there is a chapter by Anderson on the developments to date with the newest tool for colloid studies, the electron microscope. Many photographs of various colloids are included. The implications of this work for other fields are stimulating. Also, there is a chapter by Edsall on the determination of size and structure from measurements of streaming birefringence. In another chapter by Powell and Eyring the basis for flow and the behavior of long-chain molecules are discussed on theoretical grounds.

Cereal chemists will welcome the 39-page chapter on the present status of starch chemistry by Kurt Meyer. He discusses the separation of starch into its components, the chemical constitution and structural characteristics of the components, and size distribution as determined by osmotic pressure and viscosity measurements. There is a section on the fine structure of starch grains and starch paste, and one on the enzymatic degradation of starch. The last part is on the constitution, degradation, and synthesis of glycogen.

Tiselius has a chapter on his new technique of absorption analysis, and shows by an example how the method can be used for studying protein hydrolysis.

Two chapters are included on the measurement of surface areas. Emmett discusses the method of low-temperature adsorption isotherms for finely divided or porous solids, and Sullivan and Hertel develop the theory and measurements for permeability methods applied to fibers and powders.

McBain deals with detergents from the point of view of their effects in solubilization. The new and interesting use of synthetic resins as agents for ionic exchange is surveyed by Robert Myers. Important work on the constitution of inorganic gels as determined by X-ray diffraction studies is described by Weiser and Milligan. The colloid chemistry of the creaming of rubber latex is ably discussed by Van Gils and Kraay.

Changes in surface tension of solutions of various substances with time and concentration are considered by Hauser in a chapter on anomalies in surface tension of solutions.

As a whole this book, the first volume of a long-awaited series, is highly satisfactory. It is only regretted that more space is not allowed for each subject, but this is partly counterbalanced by the excellent bibliography included with each contribution. The book is recommended to all interested in the techniques and interpretations of colloid chemistry and if the succeeding volumes are of equal merit, the series will be a most valuable contribution to colloid science.

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SUPPLEMENT

THE DETERMINATION OF MOISTURE IN THE WET MILLING INDUSTRY. I. CORN

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The wet milling industry, composed of eleven refiners, process annually 80 to 100 million bushels of corn into starch, corn sirup, corn sugar, by-product feeds, and corn oil. In the wet milling process, the corn is first "steeped" in a dilute sulfurous acid solution, wherein the original moisture of the corn is increased to approximately 42% in order to soften the berry for resolution into the component parts of starch, protein, fiber, and germ. Starch is either dried as such or resuspended with water for acid hydrolysis into corn sirup or sugar. The germ is dried and expressed for its oil.

The entire process, as the name would indicate, is carried out through wet separation. All plants are "bottled up"; that is, water used in separation moves counter-current to corn in process. The water in this backward flow accumulates solubles, until it is finally treated with sulfur dioxide and used for soaking or steeping the incoming corn. This latter operation considerably increases the soluble content, and the steep water is finally removed from the system by evaporation and added to the various feeds.

In such separation, the question of losses becomes essential from the standpoint of operating efficiency. This efficiency, which is termed the "yield," is expressed as the quotient of the dry substance recovered by the dry corn substance introduced into the process. Normal losses will run between 2% and 3%. This loss has led to extended studies of losses through char used in refining, vacuum pan volatiles and entrainment, fermentation losses in processing, etc., which are fairly well known. Despite extended work in this direction, a large part of the loss was still unexplained. In terms of operation, a dry-substance loss of 2% to 3% was deemed impossible.

The entire yield picture is predicated on dry substance obtained from moisture data, and any discussion immediately reverts to whether or not the moisture data are accurate; that is, whether true moisture or relative moisture has been obtained. The controversy over moisture methods resulted in the Technical Advisory Committee of the Corn Industries Research Foundation initiating a study on the dry substance of corn sirup. This work resulted in data which showed

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that the current methods for moisture in corn sirup underestimated the true moisture in a 43° Baumé sirup by as much as 1.4%. Since a considerable part of the output of every refiner is corn sirup, the data extended the loss in yield, and renewed the controversy. As a result, the Corn Industries Research Foundation established a full-time fellowship in the laboratory of a member company to investigate all moisture methods, in order to ascertain whether true moisture was being obtained with existing methods, and if not, whether true moisture could be obtained instead of relative moisture.

Although a large number of moisture studies have been conducted on cereals and feeds, their general findings have been far from satisfactory. Halvorson (1937) concluded that it is impossible to determine the true moisture content of cereals and feed products. Exhaustive studies on the moisture of wheat and flour by Snyder and Sullivan (1924, 1925, and 1926) led to the conclusion that the moisture method must be considered as a chemical tool which can yield only relative results. Nowak and Enders (1936) state that it is not possible to make an absolute determination of water in barley, malt, and hops, since the water is always present in different types of combinations. According to Nelson and Hulett (1920), water is held with great tenacity by cereals and appreciable amounts may still be present in materials heated to a high temperature. Gortner (1938) draws the conclusion from Nelson and Hulett's work that approximately 9% of the water in wheat flour is "bound" and that this water has no appreciable vapor pressure *in vacuo* at 100°C.

Kent-Jones (1939) in his recent book, *Modern Cereal Chemistry*, clearly expresses the present trend of thought on the subject of moisture in cereals: "The determination of the amount of moisture in flour is further complicated by the fact that portions of the water are apparently joined to the solid particles in varying degrees of affinity. To use a broad generalization mentioned earlier, a portion of the moisture would appear to be more 'moisture of constitution' than 'moisture of wetness.' When cereal products are heated, loss of moisture occurs. Initially, the loss is fairly rapid, but as the heating proceeds, and the moisture content becomes reduced, it becomes more and more difficult to remove further moisture. Apparently, some of the moisture in cereals is present in the free state (described above as 'moisture of wetness') while a portion of it is intimately associated or 'bound' with the proteins, etc. (described above as 'moisture of constitution'). The driving off of the 'bound' water by prolonged heating may quite alter the nature of the material. Generally, no sharp line of demarcation can be drawn between the two classes of moisture, and the determination of moisture, therefore, becomes an empirical process in which

the conditions must be carefully controlled if comparable results are to be obtained. Fortunately for the majority of purposes for which moisture determinations are conducted, absolute results are not necessary, and any process which yields comparable results may be made to serve."

The general consensus of opinion is that the true moisture content of cereals cannot be determined and that moisture methods must be looked upon as being purely empirical. As stated by Kent-Jones above, for the majority of purposes absolute moisture results do not appear to be necessary, and, therefore, empirical methods have been found to serve the purpose quite well. This is particularly true for the wheat milling industry, where the separation of the flour from the germ, shorts, and bran is purely physical. As a result, an empirical method for ground wheat, such as the vacuum oven method at 100°C, serves equally well for its various milled products. However, the situation in the corn milling industry is more complicated. Chemical as well as physical changes occur during milling. The various by-products differ markedly from each other in their properties. Obviously, an empirical method suitable for corn would be quite unsuitable for corn sirup or gluten feed. Each corn by-product requires the use of a moisture method suitable to its properties. As a result, it would follow that not one but at least three to five empirical moisture methods might be necessary for the estimation of moisture in corn and its products. These methods could be readily devised, and their use could lead to relative results. However, and this is the point which must be stressed, the results obtained would have little value in assessing the true efficiency in terms of yield of the milling process. Empirical moisture methods cannot yield the type of data required for the corn wet milling industry.

It was decided, therefore, to re-investigate the problem of moisture in corn and its by-products in order to ascertain whether some method could be devised whereby the determination of the true moisture could be accomplished. This paper is the first of a series dealing with moisture studies of the products, with the exception of sirups and sugar, common to the wet milling industry.

The moisture method for corn now accepted as official by the U. S. Department of Agriculture for grading purposes is the jacketed water-oven method. The method requires unground corn to be dried for 96 to 120 hours in a water oven heated to the temperature of boiling water (99°–100°C). Both the Brown-Duvel moisture tester (1907) and the Tag-Heppenstall moisture meter have been calibrated to give results in agreement with this method.

Considerable evidence has accumulated, suggesting that the water-oven method underestimates the moisture content. As early as 1889, Winton found that when corn meal was dried in a current of dry hydrogen for 5 hours at a temperature of boiling water, the results obtained were about 1% higher than those obtained by drying in air in an open dish. Hopkins (1898) also used the hydrogen drying method for corn, his conditions involving the drying of ground corn for 8 hours in hydrogen at 105°C. Recently, Cook, Hopkins, and Geddes (1934) have shown that the Brown-Duvel method consistently underestimates the moisture in wheat. Snyder and Sullivan (1924, Part I) found that flour yielded on the average 1.87% less moisture when dried in air as compared to *vacuo* at 100°C. Fabris (1931) stated that the Brown-Duvel method yielded results for corn which, on the average, were low by 0.73%. He recommended the use of a turpentine distillation method.

Recently Baehr (1940) found that the drying of corn at 100°C *in vacuo* resulted in an appreciable loss of volatiles. The amount of volatiles was determined, and by subtracting this value from the total weight loss a measure of the moisture present was obtained. He found that each of his drying methods yielded a different moisture value, and that constancy in weight was unobtainable since a slight loss continued over a long drying period. His results suggested that the determination of moisture in corn was quite empirical.

Plan of Investigation

The plan first involved a complete and thorough study of the moisture content of a single homogeneous sample of corn ground to pass a 40-mesh sieve. By so doing, both the grinding and sampling error could be largely eliminated. Moreover, by using finely ground material, it was believed that the moisture problem would be somewhat simplified, inasmuch as case-hardening, which hinders the liberation of moisture from within the particles, would be reduced to a minimum. Also, the rate of moisture removal would be greater than if corn of coarser mesh was used. Later, the plan involved a study of corn ground to a coarser mesh (10) and finally work was to be conducted with whole corn. This paper deals with the results obtained with the finely ground corn (40-mesh).

Five independent approaches were made to the moisture problem, which are summarized below:

I. *Distillation Methods:* Direct water; high temperature; no vacuum.

Benzene	80°C	boiling point
Toluene	110°C	" "
Toluene-xylene (1–1 by volume)	120°C	" "
Xylene	140°C	" "

II. *Oven Methods:*
  a. Vacuum—indirect water; high temperature; high vacuum.
    70°C
    80°C
    100°C
    110°C
  b. Air—indirect water; high temperature; no vacuum.
    100°C
    100°C over P_2O_5
III. *De Bruyn:* Indirect water; low temperature; high vacuum.
    38°C
  a. Corn alone.
  b. Corn soaked in 80% alcohol and dispersed on Hyflo Filter Aid.
IV. *Extraction Methods:* Resolution into stable and unstable fraction with special treatment fitting each fraction.
V. *Reversibility Studies:* Based on the assumption that water-holding capacity is a physical constant, if decomposition or chemical alteration has not occurred.

Analytical Methods

Materials: In order to secure a representative sample of corn, the following procedure was adopted. A quart of corn was secured from each of 14 cars of approximately the same moisture content, according to government grading. From this a composite sample of approximately 15 pounds was made. This was placed in a refrigerator and when cold was first ground through an attrition mill. That passing a 40-mesh screen was run into a 3-gallon bottle. The coarse portion was again placed in the refrigerator and when cold, reground in a coffee mill. The portion passing the 40-mesh screen was run into the bottle, and that retained was returned to the refrigerator. The operation was repeated until all of the sample passed 40-mesh. The combined 40-mesh sample in the bottle was shaken periodically over a period of 10 days to insure homogeneity of sample. The material was then transferred to 4-ounce screw-top, wax-lined bottles, and returned to the refrigerator until required for use.

Distillation methods: The distillation procedure followed was essentially that outlined by Bidwell and Sterling (1925), with the exception of specially designed apparatus. The distillation flask was a 250-ml Erlenmeyer with a 40/50 "standard taper" joint. The trap was of 5-ml capacity with 40/50 and 24/40 joints, each trap having been recalibrated at milliliter intervals.

The inner surfaces of traps and condenser were treated in the following manner to avoid entrained water globules: They were first scrubbed with cleansing powder. After washing with distilled water, they were allowed to stand full of freshly prepared cleaning solution for 4 hours. They were then washed with distilled water, followed by one washing with dilute caustic (1%) followed by distilled water, and then dried under room conditions for 24 hours.

The distillation rack was a modified extraction outfit built by the Precision Scientific Company to handle the above flasks, traps, and condensers. Six hot plates were available, each with vari-heat rheostat, which has been found invaluable in securing the proper rate of distillation for precise work. Each hot plate was equipped with a small stainless steel oil bath 4 × 4 inches, filled with corn oil. The oil bath has been found highly desirable in that heat is applied from the sides as well as the bottom, which results in more even boiling and lower temperature on the plate than when the flasks rest directly on the plate.

Twenty-five to 30 g of ground corn, depending on its moisture content, was weighed into a tared flask containing 5 to 8 g of asbestos, both of which had been previously dried in an air oven at 100°C. Asbestos has been found to prevent troublesome bumping, which causes erratic distillation. Sufficient solvent to cover the corn and asbestos (75 ml) was immediately added to the flask, which was then connected to the trap and condenser. After the trap was filled with solvent, distillation was started, and was continued until a period of 24 hours showed no increase in water.

Oven methods: The standard A.A.C.C. moisture dish was used (55 × 15 mm). The duplicate samples (4.5–5.5 g) include the sampling error, since the samples for single determinations were taken from different bottles of corn. The covers of the dishes were removed during drying but were quickly replaced upon removal of the dishes from the oven. Air passing through concentrated H_2SO_4, Drierite, and P_2O_5 was used to release the vacuum in the ovens. Calcium carbide was used as the desiccant in the vacuum desiccator, 15 to 20 minutes being allowed for cooling of the samples.

Weber vacuum ovens were used. The pressure obtained ranged from 0.2 to 2.5 mm. The desired temperature was maintained within ±1.0°C. No more than 6 to 10 samples were placed in the oven at one time. For air-oven tests, an Elconap oven was used.

De Bruyn method: De Bruyn and Van Laent (1894) devised an apparatus for the drying of maltose at low temperatures under vacuum in the presence of P_2O_5. Cleland and Fetzer (1941) markedly improved the design of the apparatus, using No Lub standard taper joints throughout. It consists of two 250-ml pear-shaped flasks connected by a glass tube, 1¼ inches in diameter and 12 inches long. This apparatus is capable of retaining low vacuum (0.1 mm or better) for relatively long periods (24 to 72 hours) and has been found to be extremely satisfactory for drying corn and feeds at low temperatures under low pressure with P_2O_5 as a desiccant. The apparatus (Fig. 1) has a marked advantage over the customary vacuum desiccator

method, where the difficulty of maintaining the vacuum is always an important factor.

The corn (10–15 g) was weighed directly into a tared De Bruyn flask and the material was allowed to reach constant weight under high vacuum at a temperature of 38°C. After removal of the bulk of the water, the P_2O_5 was replaced with fresh desiccant.

Cleland and Fetzer had also shown that dispersion was an extremely important factor in the drying of sirups at low temperatures in the De Bruyn apparatus. They used Hyflo Filter Aid as a dispers-

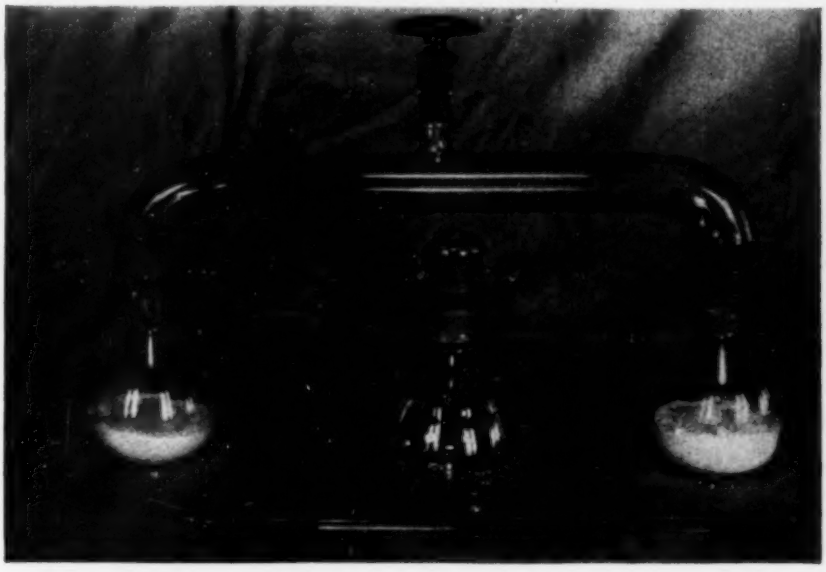

Fig. 1. The De Bruyn apparatus.

ing agent, and the sirup was intimately mixed with this material. It was thought that this technique might prove of value for corn.

As a result, the following procedure was also used: Ten to 15 g of corn was weighed into a weighing bottle and 50 to 75 ml of 80% alcohol by volume was added and the mixture allowed to stand overnight. It was then transferred to a tared de Bruyn flask containing approximately 20 g of vacuum dried (100°C) Filter Aid. The solvent was removed by placing the flasks in a vacuum oven at room temperature for 40 hours. It was thought that this procedure would result in the solution of the bulk of the corn protein, and as a consequence its finer dispersion on Filter Aid would result in a more ready loss of moisture.

The vacuums in the De Bruyn were determined daily and they were re-evacuated to 0.1 mm or less. Weighings were made at 24 to 72-hour intervals.

Extraction method: Eight to 10 g of corn was weighed into vacuum-dried (100°C) Whatman extraction thimbles contained in weighing bottles. A light plug of cotton wool was placed above the corn. The bulk of the moisture was removed from the corn by predrying the material for 48 hours at 80°C in a rapid stream of nitrogen. The corn was then extracted for 48 hours with ethyl ether. The ether-soluble fraction was taken to essential dryness on a steam bath, and was then dried to constancy in a stream of nitrogen at 75°C. The thimbles containing the ether-insoluble fraction were transferred to the weighing bottles and dried to constancy in vacuum at 100°C. By subtracting the weight of the ether extract from the total weight loss obtained in the thimbles, a measure of the moisture present in the corn was obtained.

Sampling and experimental error: In all cases, the difference between duplicates includes the sampling error, since the duplicate samples for each determination were taken from different bottles of corn. The check values for all determinations, with the exception of the oven results, are included in this paper. It will be noted that the distillation values agree within 0.10%, the De Bruyn values agree within 0.03%, and that the ether-extraction values agree within 0.06%. The SE of the duplicates from the means for the oven determination was 0.03%, the maximum difference between duplicates rarely exceeding 0.05%.

Moisture Results

Distillation results: Distillation methods have come into common use for the determination of moisture in a wide variety of products. The Brown-Duvel distillation method, based upon the principle and methods first described by Hoffman (1902), is now accepted as the official method for wheat, corn, and other cereals. The method is based on the principle of distilling the grain in a solvent immiscible with H_2O. The distilled water is collected and measured in some convenient form of measuring device. Dean and Stark (1920) were the first to develop the reflux type of apparatus, which was improved upon by Bidwell and Sterling (1925). The paper by Dean and Stark includes a comprehensive bibliography of all the work up to that date. Various immiscible solvents have been used which include commercial hydrocarbon products of varying boiling points—xylene, toluene—and recently use has been made of solvents of densities greater than water, such as carbon tetrachloride, tetrachlorethylene, and trichlorethylene.

Apparently no systematic attempt has been made to use the distillation methods for the determination of the true moisture present in cereals and feeds. The common practice has been to develop a distillation procedure against some other standard method, so that results are comparable. The Brown-Duvel, for instance, was standardized against the water-jacketed method. In their paper, Bidwell and Sterling stated that the distillation is usually complete within an hour when toluene is employed, and presented a table showing results for cereals and other substances in comparison with oven methods. Since distillation for an hour checked the oven method, it was deemed satisfactory and apparently no thought was given to whether added distillation time would give additional water or whether the oven methods represented true moisture. Thus (by an arbitrary procedure) a new technique on common products was tied to an oven method which, in turn, yielded relative moisture data.

Little if any account was taken of the fact that moisture might be retained more in one material than another and, therefore, would require additional distillation time or special experimental procedure. Thus for example the moisture data given by Bidwell and Sterling for Karo sirup is 20.3%. Fetzer and Evans (1935), using the same method but dispersing the corn sirup product on Filter Aid to make the moisture more available, obtained 27.5%. Thus, when a distillation method is standardized against another arbitrary procedure, it can only give moisture values of the same order of magnitude as the reference procedure, and may fail to indicate the true moisture content.

In this investigation a systematic use has been made of immiscible solvents of varying boiling points, carrying each distillation to completion. The moisture content of the 40-mesh corn was determined by distillation with benzene bp 80°C, toluene bp 110°C, a toluene-xylene mixture (1 : 1) bp 120°C, and xylene bp 140°C. The results obtained are shown graphically in Figure 2, and the final results are given in Table I.

It is of interest to note that the use of toluene, which boils at 30°C higher than benzene, yielded no further moisture. The solvents with higher boiling points gave a further moisture increase. However, it is well to point out that this additional increase was accompanied by the formation of an intense red color in the corn, indicating decomposition.

In the official toluene distillation method as given by the A.O.A.C. for moisture in feeds, which is essentially the method used in this investigation for the toluene procedure, it is stated that the entire process is usually over within one hour. This definitely has not been found true for corn and other feed-products used in our investigations. At the end of the three-hour period, the apparent moisture value of this

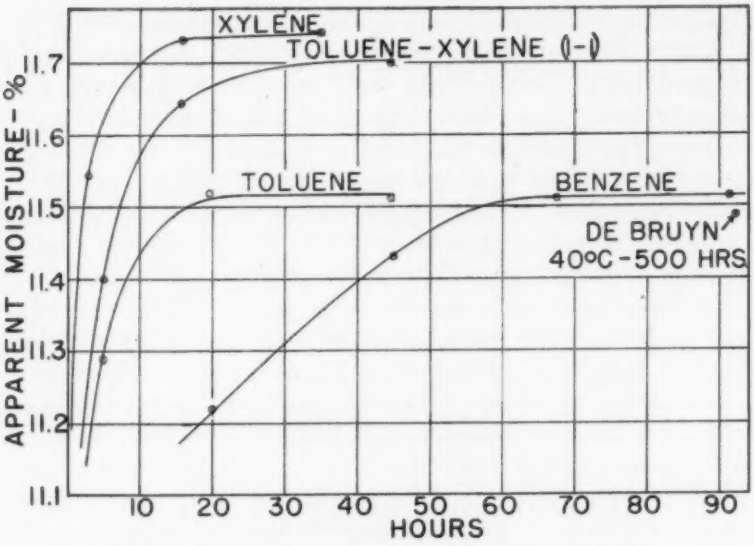

Fig. 2. Determination of moisture in corn by distillation methods.

TABLE I

DISTILLATION DATA FOR 40-MESH CORN

Solvent[1]	Length of distillation, showing period of constancy	Moisture determinations	Average
	hrs	%	%
Benzene	68–92	11.47, 11.49, 11.56	11.51
Toluene	20–45	11.49, 11.50, 11.54	11.51
Toluene-Xylene	16–45[2]	11.66, 11.74	11.70
Xylene	16–35	11.73, 11.74	11.74

[1] A correction factor of 0.02 ml is required for benzene and 0.03 ml for toluene. A factor of 0.03 ml was used for xylene and for the xylene-toluene mixture.
[2] Constancy not attained.

sample of corn by the toluene distillation method was still 0.4% too low, as compared with the final constant value. The distillation results will be discussed in a later section of this paper.

Oven results: The apparent moisture content of the corn was determined under various oven drying conditions. The corn was dried *in vacuo* at 70°, 80°, 100°, and 110°C. It was also dried in the air oven at 100°C over P_2O_5. The method used for drying the corn at 100°C over P_2O_5 was as follows: The moisture dishes were placed in the bottom of a 5-lb tin, and two glass containers containing P_2O_5 were supported above the corn samples. Two small holes were punched into the lid. Fresh P_2O_5 was used, as the conditions merited it. The complete data are shown graphically in Figure 3. The moisture value

obtained by the toluene distillation method is included in Figure 3, as well as the final value obtained by the De Bruyn procedure.

The results shown in Figure 3 clearly indicate that it is impossible to estimate the true moisture content of the corn by oven methods. Each time-temperature curve yields a different result. It might be thought that since the 100° and 110°C vacuum-oven results are in close agreement, the true moisture lies in this region. This assumption is completely erroneous, as will be shown later.

It is of interest to note that corn dried to constancy in an air oven at 100°C yields an apparent moisture value which is 1.14% lower than

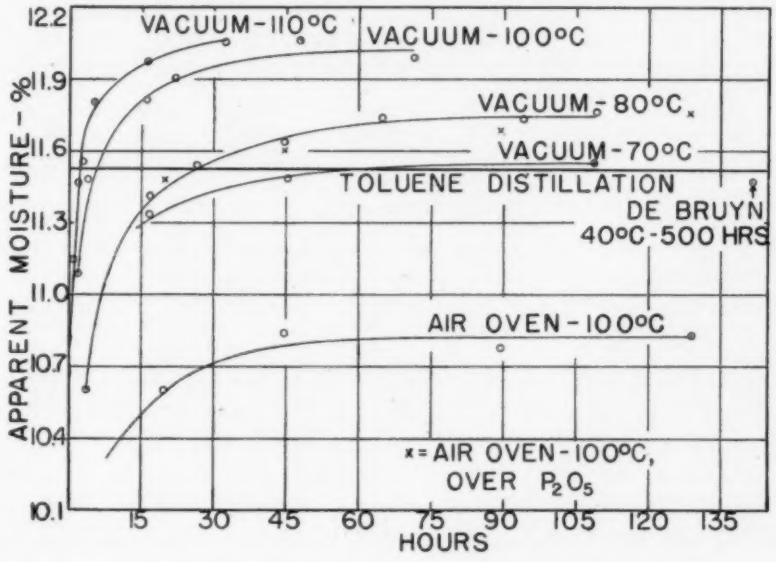

Fig. 3. Determination of moisture in corn by oven methods (40-mesh).

that obtained by drying the corn at 100°C *in vacuo*. That this difference is largely due to the ability of corn to retain water in an air oven at 100°C was proved by the fact that when the corn was dried in air over P_2O_5 at 100°C, this difference between air and vacuum drying largely disappeared, the difference being reduced to only 0.21%. If vacuum-dried corn is replaced in an air oven at 100°C, it actually adsorbs moisture, thus indicating the marked affinity of this material for water even at high temperatures (100°C). These results cast great doubt on the official water-jacketed-oven method, as a measure of true moisture.

The oven results will be referred to again in relation to the other data.

The De Bruyn method: The moisture content of the corn was determined by drying it over P_2O_5 under a high vacuum at a low temperature (38°C). Other samples of corn were first "soaked" in alcohol and dispersed on Filter Aid before undergoing the same drying treatment. Both sets of De Bruyns, as shown in Table II, reached con-

TABLE II

THE DE BRUYN DRYING OF 40-MESH CORN—APPARENT MOISTURE

Length of drying	Corn			Corn +80% alcohol +Filter Aid		
	Duplicates		Average	Duplicates		Average
	38° C					
hrs	%		%	%		%
275	11.32	11.33	11.32	11.31	11.30	11.30
600	11.45	11.50	11.48	11.47	11.45	11.46
700	11.45	11.51	11.48	11.49	11.45	11.47
800	11.45	11.49	11.47	11.46	11.45	11.46
	50° C					
An additional 100 hours	11.45 to 11.49		11.47	11.46 to 11.45		11.46

stancy at the same moisture value, 11.46% to 11.47%. The results obtained indicated that the alcohol dispersion procedure resulted in no advantage over the untreated corn, either in the rate of moisture removal or in the final value obtained.

Within 275 hours of drying, the corn samples reached a moisture value within 0.20% of that obtained by benzene or toluene distillation. Constancy was reached within 600 hours, after which no further weight change occurred during an additional 200 hours of drying. Further drying for another 100 hours at 50°C caused no additional moisture loss. The average constant value for the four determinations was 11.46%, which is within 0.05% of that obtained by distilling corn to constancy with benzene or toluene.

The connecting tubes of the De Bruyns were cleaned with cleaning solution prior to use, and if any volatiles had distilled over during drying, it should have been quite observable. Possibly a trace was present in two of the tubes, but the amount was unappreciable. The results show that corn can be dried at low temperatures (38°C) to yield a moisture value essentially equal to that obtained by distilling corn to constancy with benzene or toluene. These results will be referred to again.

Ether extraction method: Baehr (1940) found that volatiles are given off from corn during vacuum drying at elevated temperatures. To correct for the volatiles, he quantitatively determined the amount given off and subtracted this value from the total weight loss during drying. The volatiles were trapped in a receiver surrounded by dry ice. The apparatus was necessarily somewhat elaborate, and in view of the small quantity of volatiles given off, a large sample of corn was required. As a result, the method is time-consuming.

A simpler method was chosen which would still account for the volatiles, but which would not require the use of flasks, connecting tubes, dry ice, large samples, etc. The method consisted in first extracting the volatiles from the corn and then drying the ether-soluble and ether-insoluble fractions separately to constancy by appropriate methods. Baehr had found that the volatiles were soluble in ether, and therefore this solvent was used for the extraction. The procedure used is given in the section under "Analytical Methods." The data are given in Table III.

TABLE III

ETHER EXTRACTION METHOD FOR THE DETERMINATION OF MOISTURE IN CORN

Ether-soluble fraction, % DS 75°C-stream of nitrogen			Ether-insoluble fraction, % DS 100°C *vacuo*			Moisture, %		
Hrs. of drying	Determinations (1)	(2)	Hrs. of drying	Determinations (1)	(2)	Determinations (1)	(2)	Av.
	%	%		%	%	%	%	%
1	4.67	4.87	40	83.70	83.55	11.63	11.58	11.60
3	4.68	4.87	60	83.69	83.56	11.63	11.57	11.60

A second determination carried out by similar methods yielded a moisture content for the sample of corn of 11.56%. By removing the volatiles prior to drying, the apparent moisture content of the corn was reduced from 12.06% (vacuum drying at 100°C of corn direct) to 11.58%. This latter value is in fair agreement with the moisture results obtained by benzene or toluene distillation (0.07%) and with the De Bruyns (0.12%). Inasmuch as it is well recognized that ether is incapable of removing the total "fat" from corn, the slightly higher moisture results obtained by this procedure can, in all probability, be attributed to the small quantity of volatiles remaining in the ether-extracted material.

That volatiles are present in the ether extract of corn is readily apparent from Table IV. Upon completion of drying the ether extract to constancy in nitrogen at 75°C, the ether extracts were dried further in air at 100°C, followed by drying *in vacuo* at 100°C.

TABLE IV

THE EFFECT OF DRYING ON THE ETHER EXTRACT OF CORN

Drying method	Ether-soluble extract, % DS	
	Determinations (1)	(2)
	%	%
75°—N_2—3 hrs	4.68	4.87
100°C—air—2 hrs	4.56	4.76
4 hrs	4.66	4.85
26 hrs	4.52	4.62
100°C—*vacuo*—20 hrs	4.30	4.48

A weight loss first occurred in the air oven at 100°C, followed by a weight gain indicating oxidation of the extract. This was noted by Hopkins (1898). Continued drying of the extract at 100°C resulted in a considerable weight loss, which became very marked when the material was dried for an additional 20 hours *in vacuo* at 100°C. An over-all weight loss of 6.5% to 7.4% of the extract is indicated, and these values are probably low since a weight gain also occurred during the drying because of oxidation. If the vacuum-oven solids values of the ether extract are used in place of the values obtained at constancy in N_2 at 75°C, an apparent moisture value of 11.98% is obtained for the corn, which is a value corresponding closely to the results obtained by drying corn directly in the vacuum oven at 100°C.

The results obtained by this experiment clearly show that volatiles are given off during the drying of ground corn in vacuum at elevated temperatures. They also indicate that the marked variability in the oven data is attributable to the presence of volatile products.

Reversibility studies: The procedure for the determination of moisture in cereals, which has been termed the reversibility method, was first applied to gluten meal, and for this reason only the basis for the method will be given here together with the results obtained for corn. A fuller description covering its usage is given in a paper dealing with gluten meal.

It has been known for a long time that completely dehydrated biological products exhibit a hysteresis effect on transfer to their original storage condition. However, Urquhart and Eckersall (1930) and Pidgeon and Maass (1930) have shown that cellulose which contains 1% of moisture may lose it reversibly. In other words, let us suppose that cellulose is stored over H_2SO_4 and also over P_2O_5, and that under the former condition the cellulose still retains 1% of moisture, while under the second it is completely dried. According to the findings of the above investigators, the moisture that is lost on transfer of the cellulose from storage over H_2SO_4 to storage over P_2O_5 is completely regained on retransfer of the cellulose back to the H_2SO_4 storage

conditions. Obviously, if the cellulose were to undergo decomposition while being dried over P_2O_5, this complete reversibility could not take place. These results offer the foundation for the reversibility method.

Relatively mild drying conditions are sufficient to remove all but the last 1% to 2% of moisture from cereal products. This last final increment is held with great tenacity as indicated by Table V.

TABLE V

WATER RETAINED BY THE PRODUCT WHEN IN EQUILIBRIUM WITH AIR AT 100°C[1]

Product	Water retained
	%
Wheat flour	1.9 (Snyder and Sullivan, 1924, Part I)
Corn (40-mesh)	0.7
Corn (10-mesh)	1.0
Gluten meal (20-mesh)	0.5
Starch	0.9
Dextrin	0.4

[1] These values will fluctuate with the humidity.

In order to effect the removal of this final increment of moisture, it has become common practice to use vacuum ovens or higher-temperature air ovens. These methods, however, offer the possibility of dry-substance loss through volatilization or decomposition, and as a consequence it is always a problem to determine at just what point the total moisture is removed and also to determine whether this total loss is caused solely through moisture loss. An attempt was made to clarify this problem by utilizing the thought that the last increment of water may be removed reversibly.

The results obtained with cellulose were applied to the cereal moisture problem. A control sample of the cereal or feed was dried under conditions which eliminated any possibility of decomposition and which permitted the material to retain roughly 1% of moisture. Other samples were dried for various periods under drying conditions sufficient to remove the total moisture. These samples were then replaced in proximity to the controls, under which conditions the more completely desiccated samples readsorb moisture. Samples which adsorb sufficient moisture to yield a final apparent moisture value equal to the control are considered to be unchanged by the drying process. Those samples which are decomposed during the more drastic drying treatment, through the loss of volatiles or other causes, are unable to attain this similar equilibrium value.

There is an obvious fault in this reasoning which would lead to erroneous data with regard to this procedure—namely, the loss of volatiles or slight decomposition might alter the primary adsorptive power of the cereals. Normally, it would be necessary to prove or

disprove this possibility, but in view of the obtained data and the close relation of the moisture values obtained by this method to the other moisture methods used, it is believed that this possibility can be neglected.

In applying the reversibility method to a particular product, some experimentation is necessary to find the most suitable conditions. Since the method is based on the sorptive capacity of the cereal, it is essential that the material must be in a fine state of subdivision. Samples used for the moisture determination may be dried at any desired temperature and pressure by the usual oven procedures. Simultaneously, control samples are dried under conditions which permit the material to retain roughly 1% of moisture. In certain cases, when the product is stable, this can be accomplished by using the air oven at 100°C. In others, as with corn, the same conditions may be unsuitable since corn contains considerable fat, some of which is volatile and also readily oxidizable. The reversibility studies with corn were first carried out as follows:

The corn samples were dried for varying periods *in vacuo* at 100°C (Fig. 3), remoistened (8 ml H_2O to 4–5 g of corn), and the excess water was removed in the vacuum oven at room temperature. The control samples were given a similar moistening treatment. All samples were then carefully repulverized and allowed to reach complete equilibrium *in vacuo* at 40°C, which was largely attained within 200 hours and completely by 340 hours. The results given in Table VI are typical of

TABLE VI

REVERSIBILITY MOISTURE METHOD FOR CORN

Control	A Apparent moisture	B Reversible moisture value, 340 hrs at 40°C	C Adsorption A–B	D Decomposition B—10.41%	E Calculated true moisture A–D
	%	%	%		%
Vacuum oven—40°C					
340 hrs	10.41	10.41	—	—	—
Vacuum oven—100°C					
2 hrs	11.09	10.39	0.70	—	—
4 hrs	11.48	10.53	0.95	0.12	11.36[1]
16 hrs	11.82	10.74	1.08	0.33	11.49
22 hrs	11.90	10.82	1.08	0.41	11.49
48 hrs	12.06	11.00	1.06	0.59	11.47
72 hrs	11.98	10.91	1.07	0.50	11.48
					Av. 11.48

[1] This value omitted from the average.

those obtained for the last three weighings (samples reweighed every 48 hours). The data are shown in a somewhat different form in Table VII.

TABLE VII

DATA OF TABLE VI REARRANGED

Vacuum oven—100°C	Loss in weight on transfer of corn dried to equilibrium in the vacuum oven at 40°C (10.41%) to the vacuum oven at 100°C	Regain in weight on replacement of 100°C vacuum oven samples to the vacuum oven at 40°C	Loss in recovery
hrs	%	%	%
2	0.68	0.70	+0.02
4	1.07	0.95	0.12
16	1.41	1.08	0.33
22	1.49	1.08	0.41
48	1.65	1.06	0.59
72	1.57	1.07	0.50

As shown in the tables, the two-hour drying period in the vacuum oven at 100°C resulted in no irreversible change as indicated by its complete recovery on retransfer to the vacuum at 40°C. Samples dried for longer periods, however, do not exhibit this complete reversibility. If complete reversibility is attained, the loss in weight should exactly balance the regain in weight, and where this is not attained, it might be attributed to a change in the adsorptive capacity of the heated samples or to the loss of volatiles or some other form of decomposition. It would be expected that with continued drying *in vacuo* at 100°C, a point would be reached where the total moisture is removed and additional heating results only in a dry-substance loss. If the adsorptive capacity of the dried products remained unchanged, they should be able to re-adsorb water to a degree dependent on their extent of drying. As shown above, those samples dried for 16 hours or longer at 100°C regain essentially the same weight; *i.e.*, they adsorb equivalent quantities of water. The constancy obtained, notwithstanding the fact that some of the samples were dried for an additional 56 hours, indicates that the adsorptive capacity was not affected. The data therefore show that the difference between the weight-gain and weight-loss values must be attributable to some irreversible loss during the vacuum drying in the oven at 100°C. The extent of this loss is shown in column D of Table VI, and when these values are subtracted from the apparent moisture results obtained in the vacuum oven at 100°C, it is found that the calculated true moisture values (column E) are in agreement for those samples dried for 16 hours or longer. Moreover, the results obtained by this reversibility method are in agreement with the moisture results obtained by the distillation methods, the De Bruyn procedure, and the ether extraction method.

A second reversibility study was conducted with several refinements. It had been found previously that an initial "wetting" is

essential in order to make all the samples comparable if vacuum-dried materials are required to adsorb considerable moisture. During the removal of the excess water, the possibility of changes, enzymatic or otherwise, is present. Work has shown that satisfactory results are obtainable without the need of first "rewetting" the material if the moisture differential between the apparent and reversible moisture values does not exceed 0.4% to 0.8%. In the second experiment, therefore, reversibility conditions were used which dispensed with this possible source of error. Since corn loses volatiles if the re-drying conditions are carried out *in vacuo* at more elevated temperatures (60°–80°C), the second study was carried out under atmospheric pressure. Work has shown that the use of an air oven at 60°–80°C is not satisfactory since humidity variations from day to day result in continued weight fluctuations. Moreover, the possibility of oxidation is present and the samples take up too much moisture. With these considerations in mind, the following conditions were used:

The corn samples dried *in vacuo* at 110°C were replaced in the Weber oven at 70°C. A constant stream of dry nitrogen was passed through the oven. By so doing, both humidity fluctuations and the possibility of oxidation were eliminated. Weighings were made from time to time and the results of typical weighings are given in Table VIII. It can be observed that the results agree admirably well with those obtained in the first study.

TABLE VIII

REVERSIBILITY MOISTURE METHOD FOR CORN

	A Apparent moisture	B Reversible moisture value	C Adsorption A–B	D Decomposition B—11.14%	E Calculated true moisture A–D
hrs	%	%	%	%	%
Control[1]	11.14	11.14	—	—	—
Vacuum oven—110°C					
1¾	11.46	11.22	0.24	0.08	—
2½	11.55	11.27	0.28	0.13	—
4	11.74	11.38	0.36	0.24	11.50
5	11.80	11.48	0.32	0.34	11.46
32	12.05	11.74	0.31	0.60	11.45
					Av. 11.47

[1] Samples dried for 96 hours at 70°C and for an additional 60 hours at 80°C in a stream of nitrogen.

Discussion

The moisture content of a single sample of corn ground to pass a 40-mesh sieve was determined by five different methods. Various modifications of each method were used. The complete data are summarized in Table IX, from which it can be observed that the ob-

TABLE IX

SUMMARY OF MOISTURE DATA FOR 40-MESH CORN

Moisture method	Conditions	Length of drying showing period of essential constancy	Final apparent moisture value
		hrs	%
Distillation	Benzene—bp 80°C	68– 92	11.51
	Toluene—bp 110°C	20– 45	11.51
	Xylene-Toluene—bp 120°C	16– 45[1]	11.70
	Xylene—bp 140°C	16– 35	11.74
Oven drying	Vacuum—70°C	46–110[1]	11.55
	80°C	65–110[1]	11.77
	100°C	48– 72[1]	12.06
	110°C	16– 32[1]	12.05
	Air—100°C	45–130	10.84
	Air—P_2O_5—100°C	20–130[1]	11.77
De Bruyn	Corn alone—38°C	650–850	11.48
	Corn alone—50°C	Additional 100	11.47
	Corn with alcohol—38°C	650–850	11.46
	Corn with alcohol—50°C	Additional 100	11.46
Extraction method	(1) Ether extract—75°C—N_2	1– 3	11.60
	Ether-insoluble—100°C—*vacuo*	18– 36	
	(2) Ether extract—75°C—N_2	2– 6	
	Ether-insoluble—100°C—*vacuo*	24– 48	11.56
Reversibility studies	(1) Corn predried—100°C—*vacuo*	—	11.48
	(2) Corn predried—110°C—*vacuo*	—	11.47

[1] Complete constancy not obtained.

tained moisture values range from 10.84% to 12.06%. The air-oven results (10.84%) are undoubtedly low, as shown by the fact that when the corn was dried over P_2O_5 in the air oven at 100°C, the moisture value obtained was 11.77%. Therefore the values deemed significant fall within the moisture range of 11.46% to 12.06%.

The results obtained by the ether extraction method clearly indicate that as much as 7% of the dry substance contained in the ether extract of corn is volatile *in vacuo* at 100°C. This percentage is equivalent to 0.45% of the original weight of the corn. By subtracting this percentage from the moisture values obtained by drying the whole corn *in vacuo* at 100° or 110°C, the obtained moisture range is reduced to include values within 11.46% to 11.61%. The moisture results obtained by the benzene or toluene distillation method, by the De Bruyn method of drying the corn under high vacuum at 38°C by the ether-extraction method, and by the reversibility method all fall within this relatively small moisture range.

The benzene and toluene distillation methods yielded identical results (11.51%) but immiscible solvents of higher boiling points caused a further increase in water obtained. This increase was

accompanied by an intense red color formation in the corn, which clearly suggests decomposition. By drying the corn to constancy *in vacuo* at 38°C over P_2O_5, a moisture value of 11.46% was obtained. Further drying at an elevated temperature of 50°C resulted in no further moisture loss. The reversibility method, which has yielded very consistent data, indicated a calculated moisture value of 11.48%. These three diverse procedures all yielded results which fall within the very narrow moisture range of 11.46% to 11.51%.

The ether extraction method yields somewhat higher moisture values (11.56% to 11.60%). However, as pointed out previously, this method should tend to yield somewhat higher results since it is well known that ether does not extract the total "fatty" substances from corn. As a result the extracted material still contains a small percentage of volatiles, which may be expected to volatilize during the vacuum drying at 100°C to constancy. The final conclusion, which the results indicate clearly, is that the true moisture value for this sample of 40-mesh corn under test lies within the narrow range of 11.46% to 11.55%. The results of these experiments show that the true moisture content of corn can be determined.

This conclusion is at variance with that obtained by all previous investigators in the moisture field, who state that it is impossible to determine the true moisture content of cereals and feeds. The moisture method has been considered to be purely empirical. Much has been said with regard to "free" in contradistinction to "bound" water in cereals. Previously it was concluded that this "bound" water could not be differentiated from "water of constitution." A paper published by Nelson and Hulett (1920) has given strong support to the belief that water is held with great tenacity upon colloidal surfaces, and that appreciable amounts of water may still be present on materials heated to a high temperature. Gortner (1938) concluded from Nelson and Hulett's data that wheat flour contains approximately 9% of its total moisture in the "bound" form and that this water has no appreciable vapor pressure *in vacuo* at 100°C. Nelson and Hulett had shown that the apparent moisture content of a sample of flour dried at 100°C *in vacuo* was 10.80% and that the probable true moisture content was 11.80%, a difference of 1.0%. However, it must be pointed out that the flour was heated for only 4 hours at 100°C in a glass tube of narrow diameter, which led through a long capillary tubing to the receiver. Under ideal vacuum-oven drying conditions, it requires approximately 16 hours at 100°C to dry flour to constancy. With the restricted apparatus used by Nelson and Hulett, this period may have required lengthening to 100 hours. The obtained moisture value of 10.80% is not indicative of the true equilibrium value at

100°C and therefore the "bound" figure value calculated by Gortner for wheat flour is open to considerable doubt. From Nelson and Hulett's data, the present authors have no reason to believe that the calculated moisture value of 11.80% could not have been obtained if the flour had been heated for a sufficient length of time at 100°C. There is no reason to conclude from their results that temperatures in excess of 100°C are required to remove the moisture from cereals and feeds when dried *in vacuo*.

In the moisture study here reported, the conditions have been very diverse. Distillation solvents which varied by 30°C in boiling points yielded the same moisture result. The drying of corn over P_2O_5 at 38°C yielded a value essentially the same as that obtained by the use of an immiscible solvent boiling at 110°C. By drying the ether extract at 75°C in N_2 and the ether-insoluble fraction at 100°C in vacuum, results were obtained which were in good agreement with either the De Bruyn or distillation methods. The reversibility method, which appears to be based on sound premises, offers strong contributory data to that obtained by the other methods. The results clearly indicate that the moisture of corn can be differentiated from "water of constitution," and that it is unnecessary to rely on empirical methods for its estimation.

Summary and Conclusions

The moisture content of a sample of corn ground to pass a 40-mesh sieve was determined by five different methods, which included oven methods, distillation methods, drying under high vacuum over P_2O_5, an ether extraction procedure by which the dry substances of the ether-soluble and ether-insoluble fractions were determined separately, and finally by a newly introduced procedure termed the "reversibility" method.

Distillation of corn with benzene or toluene yielded similar results (11.51%), while the use of solvents of higher boiling point resulted in higher moisture values. This increase was accompanied by an intense red color formation in the corn.

The oven methods yielded widely varying results, which ranged from the low of 10.84% for the air oven at 100°C (130 hours) to the high of 12.05% for the vacuum oven at 100°C (72 hours). It was demonstrated that the corn dried in the air oven still contained moisture, as shown by the fact that when the material was placed in the air oven at 100°C over P_2O_5, a moisture value of 11.77% was obtained.

Corn can be dried to constancy at low temperatures (38°–50°C) *in vacuo* over P_2O_5 to yield a moisture value essentially equal to that obtained by the benzene or toluene distillation method (within 0.05%).

By first extracting corn with ethyl ether and then determining the dry substance in each fraction separately, a moisture value of 11.56% to 11.60% was obtained. These moisture values may be expected to be somewhat high since ether is generally considered to be unable to extract all the "fat" from corn. The ether extract of corn contains 6.5% to 7.4% of material which volatilizes *in vacuo* at 100°C. The results of this study unmistakably indicated that the major cause for the variability in the vacuum oven results can be attributed to the ether extract of corn.

The determination of the moisture content of the corn by the reversibility procedure yielded calculated moisture values ranging from 11.45% to 11.50%, which are in close agreement with the values obtained by benzene or toluene distillation (11.51%), by the De Bruyn method (11.46%), and by the ether extraction method (11.58%). The results of this investigation lend strong support to the reversibility method which is based on the assumption that during drying, no change occurs to the primary water-combining properties of the material.

The results of this study would clearly indicate that the *true* moisture content of corn can be determined.

Literature Cited

Baehr, H. A.
1940 Progress reports on the moisture of corn. On file at Corn Industries Research Foundation, 5 East 45th Street, New York City.
Bidwell, G. L., and Sterling, W. F.
1925 Preliminary notes on the direct determination of moisture. Ind. Eng. Chem. **17**: 147–149.
Brown, E., and Duvel, J. W. T.
1907 A quick method for the determination of moisture in grain. U. S. Dept. Agr. Bur. Plant Industry, Bul. No. 99.
Cleland, J. E., and Fetzer, W. R.
1941–42 Determination of moisture in sugar products. Ind. Eng. Chem. (Anal. Ed.) **13**: 858–860; **14**: 27–30; **14**: 124–129.
Cook, W. H., Hopkins, J. W., and Geddes, W. F.
1934 Rapid determination of moisture in grain. Can. J. Research **11**: 264–289.
Dean, E. W., and Stark, D. D.
1920 A convenient method for the determination of water in petroleum and other organic emulsions. J. Ind. Eng. Chem. **12**: 486–490.
De Bruyn, Lobry C. A., and Van Laent, F. H.
1894 Maltose and its anhydride. Rec. trav. chim. **13**: 218–222.
Fetzer, W. R., and Evans, J. W.
1935 Baumé-purity-moisture tables for corn sirup. Ind. Eng. Chem. (Anal. Ed.) **7**: 41–43.
Fabris, U.
1931 Determination of moisture in corn. Z. Untersuch. Lebensm. **61**: 345–347.
Gortner, R. A.
1938 Outlines of biochemistry. John Wiley & Sons, Inc., New York.
Halvorson, H. A.
1937 Report on moistures. J. Assoc. Off. Agr. Chem. **20**: 435–438.
Hoffman, J. F.
1902 Verfahren und Apparat zur Bestimmung des Wassergehaltes in Organishen Substanzen. Z. angew. Chem., p. 1193, Berlin.
Hopkins, C. G.
1898 The chemistry of the corn kernel. Ill. Agr. Exp. Sta. Bul. 53.

Kent-Jones, D. W.
1939 Modern cereal chemistry. Northern Publishing Co., Ltd., Liverpool.
Nelson, O. A., and Hulett, G. A.
1920 The moisture content of cereals. Ind. Eng. Chem. **12**: 40–45.
Nowak, G., and Enders, C.
1936 The determination of water in barley, malt, and hops. Chem. Zentr. **11**: 202–203.
Pidgeon, L. M., and Maass, O.
1930 The adsorption of water by wood. J. Am. Chem. Soc. **52**: 1053–1069.
Snyder, H., and Sullivan, B.
1924 Determination of moisture in wheat and flour, Part I. Ind. Eng. Chem. **16**: 741–744. Part II, **16**: 1163–1167.
1925 Determination of moisture in wheat and flour, Part III. Ind. Eng. Chem. **17**: 311–314.
1926 Determination of moisture in wheat and flour, Part V. Ind. Eng. Chem. **18**: 272–275.
Urquhart, A. R., and Eckersall, N.
1930 The moisture relations of cotton. VII. A study of hysteresis. J. Textile Inst. **21**: T499–T510.
Winton, A. L.
1889 Conn. Agr. Exp. Sta. Report, p. 187.

THE DETERMINATION OF MOISTURE IN THE WET MILLING INDUSTRY. II. CORN

L. SAIR[1] and W. R. FETZER[2]

The results reported in Part I have proved that it is possible to determine the true moisture content of corn. Four diverse procedures, which clearly distinguished moisture from volatiles and decomposition, yielded moisture results which were in agreement.

This conclusion was based on the study of a single finely ground (40-mesh) homogeneous sample of corn. In practice, a coarser grind, such as a 20- or 10-mesh, would be much more desirable from the standpoint of preparation of sample. It was recognized that such a grind would produce small, hard grits from which moisture would be removed more slowly and perhaps not completely by some of the methods, such as the De Bruyn and benzene distillation, used successfully for the finely ground (40-mesh) material. Therefore it was decided to conduct a moisture study with a coarser grind—even greater than might be chosen for a laboratory method. For this purpose, a 10-mesh was chosen.

The first section of this paper is devoted to a moisture study of 10-mesh corn. The second section deals with the recommended official reference method for moisture in corn which was accepted by the Corn Industries Research Foundation (C. I. R. F.). The third shows the relation of the C. I. R. F. moisture method to the present official methods, and the final section shows the relation of the C. I.

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R. F. moisture method to the methods now in common use by the members of the industry.

The Moisture of 10-Mesh Corn

For test purposes, a composite sample of corn was obtained by sampling ten cars of similar grading. Preparation of the sample and methods used followed the procedure described in Paper I.

TABLE I

DISTILLATION DATA FOR 10-MESH CORN—PERCENT MOISTURE

Benzene (200 hrs distillation)	Toluene (140 hrs distillation)
%	%
14.26	14.08
14.23	14.25
14.08	14.10
14.15	14.28
14.13	14.23
Av 14.17	Av 14.19

Distillation results: The moisture content of the 10-mesh corn was determined by distillation with benzene and toluene, and the results obtained are shown graphically in Figure 1, and the final values are shown in Table I.

The results indicate that both benzene and toluene yield essentially the same moisture value for 10-mesh corn. It will be observed that individual determinations for each method show considerably more variation than was obtained previously with 40-mesh material. This probably results from lack of homogeneity in the 10-mesh sample and failure to remove the last traces of moisture from the hard grits.

When 40-mesh corn was used, it was found that the benzene and toluene distillation methods reached constancy within 45 and 16 hours, respectively. With the 10-mesh corn, these periods were increased to 130 and 100 hours, respectively, a three- to six-fold increase. Granulation has a marked effect on the rate of water removal. This will be shown by experiments later in the paper.

The toluene distillation method is official for grain and stock feeds. The method states that the entire process is usually completed within an hour. At the end of two hours, the moisture value obtained for the sample of 10-mesh corn used above was 12.60%, a value which is still 1.59% lower than that obtained upon completion of the distillation. It would appear that an arbitrary assignment of an hour for distillation time can lead to erroneous results and the prerequisite time can be obtained only by a study of the specific material under investigation.

Oven results: The moisture content of the corn was determined by drying the material *in vacuo* at 80° and 100°C, and by the air-oven method at 100°C. The data are shown graphically in Figure 1.

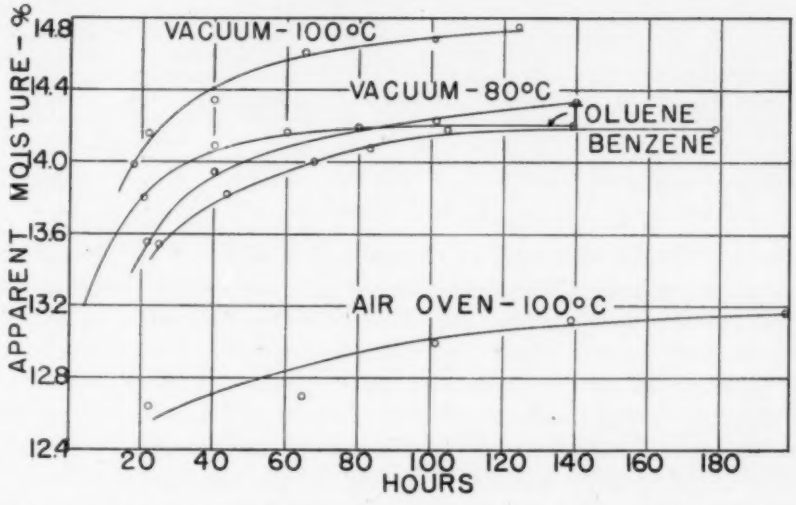

Fig. 1. Determination of moisture in corn by the oven and distillation methods (10-mesh).

The results obtained are substantially the same as those previously obtained with the finely ground corn, with the difference that the rate of moisture loss in all cases was markedly lessened. Whereas it required only 4 hours *in vacuo* at 100°C to dry corn (40-mesh) to a value equal to that obtained by toluene distillation, it required 22 hours for the 10-mesh corn.

The 100°C air-oven values were again markedly low as compared to the vacuum-oven results, a difference of 1.59% being obtained. In the previous study with 40-mesh corn, the difference obtained by the two methods was 1.22%. Apparently the coarser the granulation, the greater the differential. This is of importance since it is well to bear in mind that the standard official moisture method for corn involves the drying of whole corn in a water-jacketed air oven.

De Bruyn results: Ten-mesh corn was dried alone and in admixture with alcohol and Hyflo Filter Aid in De Bruyns at 50°C. The results in Table II show a considerable contrast to those previously obtained with the 40-mesh corn.

Within 275 hours at 38°C, the moisture values obtained with the 40-mesh corn were within 0.2% of those obtained by benzene or toluene distillation. Also, the alcohol treatment resulted in no advantage over the untreated corn. With 10-mesh corn at the end of 300 hours of drying at 50°C, the moisture values for the corn dried

TABLE II

THE DE BRUYN DRYING OF 10-MESH CORN—PERCENT MOISTURE

Length of drying	Corn			Corn +80% alcohol +Filter Aid		
	Duplicates		Average	Duplicates		Average
hrs	%	%	%	%	%	%
300	13.42	13.43	13.42	13.86	13.91	13.88
400	13.49	13.54	13.52	13.90	13.93	13.92
500	13.56	13.59	13.58	13.96	13.96	13.96
700	13.72	13.64	13.68	14.00	14.04	14.02
900	13.79	13.72	13.76	—	14.10	14.10

alone and for the alcohol-treated corn were 0.76% and 0.30% too low. Further drying up to 900 hours reduced this differential to 0.43% and 0.09%, respectively. The use of alcohol for the solution of the proteins and their dispersion in Filter Aid are very advantageous with coarsely ground corn. These results demonstrate again the important part played by granulation during the drying of materials at low temperatures. Although the untreated samples had not reached constancy even after prolonged drying, it appears doubtful whether sufficient moisture would be lost with further continued drying to equal the values obtained by the distillation methods. Rather it seems as though a constancy would be reached in which the corn would appear to be at equilibrium with the desiccant, but still containing entrapped water. As a result, a false equilibrium would be obtained which, if not recognized, would lead to false conclusions. It might be concluded that the lower moisture values are due to "bound" water or to the water-binding capacity of the corn, rather than to the true reason, which is "case-hardening" or the unavailability of the water to the desiccant.

After 900 hours of drying, the alcohol-treated corn yielded a value within 0.09% of the toluene distillation. Inasmuch as constancy was not reached, these results add further confirmation to the belief that corn can be dried *in vacuo* at low temperatures over P_2O_5 to yield values essentially similar to those obtained by distillation.

Ether extraction method: The moisture content of the 10-mesh corn was determined by the ether extraction method, as previously described. The results are given in Table III.

The ether extraction method yields a calculated moisture content of 14.28%, in contrast to the value of 14.75% obtained by drying the whole corn directly *in vacuo* at 100°C. These results again demonstrate the error involved by vacuum drying of corn at 100°C through the loss of volatiles. The ether extraction value (14.28%) is reasonably close to that obtained by toluene distillation (0.09%).

TABLE III

EXTRACTION METHOD FOR THE DETERMINATION OF MOISTURE IN 10-MESH CORN

Ether-soluble fraction, % DS 75°C—stream of nitrogen			Loss in weight of the ether-insoluble fraction—100°C—*vacuo*			Moisture		
Hrs of drying	Determinations		Hrs of drying	Determinations		Determinations		Av
	(1)	(2)		(1)	(2)	(1)	(2)	
	%	%		%	%	%	%	%
2.5	3.90	3.83	40	18.17	18.09	14.27	14.26	14.26
3.5	3.90	3.83	64	18.18	18.11	14.28	14.28	14.28

As found previously for 40-mesh corn, this method tends to yield somewhat higher results, which it is believed can be attributed to the presence of traces of volatiles remaining in the extracted corn.

Reversibility: Considerable difficulty was experienced in the application of the reversibility method to coarsely ground corn (10-mesh). As shown in the section on De Bruyn methods, coarsely ground corn tends to reach a false equilibrium when dried under mild conditions, because of the unavailability of the "entrapped" water to the desiccant. As a result, the De Bruyn method on the untreated sample underestimates the moisture content.

It has been pointed out previously that reversibility experiments must be conducted under drying conditions which eliminate any possibility of decomposition. Otherwise the check samples of corn would undergo some change. However, if the check samples of 10-mesh corn are dried under the conditions essential for a proper reversibility study, we then find that the material behaves in a manner similar to that exhibited by the corn in the De Bruyns. An equilibrium condition is reached with continued drying but the moisture value obtained, if accepted, would lead to false conclusions. The factor of granulation is an inhibiting influence, as the moisture value is much lower than would normally be obtained. As a result, the check moisture value cannot be used as a basis of comparison against the previously heat-treated samples used for moisture determinations. It has therefore been found impossible to draw any conclusions from the studies conducted with coarsely ground corn without first making an added assumption.

The reversibility studies with 40-mesh corn were carried out without encountering the difficulties mentioned above. Three different studies were conducted, and in each case it was found that the ratio of water to volatile loss (decomposition) was essentially the same; *i.e.*, it was found that when the apparent moisture value of the corn equaled the toluene distillation value, decomposition equivalent

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THE DE BRUYN DRYING OF 10-MESH CORN—PERCENT MOISTURE

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	Duplicates		Average	Duplicates		Average
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400	13.49	13.54	13.52	13.90	13.93	13.92
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700	13.72	13.64	13.68	14.00	14.04	14.02
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After 900 hours of drying, the alcohol-treated corn yielded a value within 0.09% of the toluene distillation. Inasmuch as constancy was not reached, these results add further confirmation to the belief that corn can be dried *in vacuo* at low temperatures over P_2O_5 to yield values essentially similar to those obtained by distillation.

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Hrs of drying	Determinations		Hrs of drying	Determinations		Determinations		Av
	(1)	(2)		(1)	(2)	(1)	(2)	
	%	%		%	%	%	%	%
2.5	3.90	3.83	40	18.17	18.09	14.27	14.26	14.26
3.5	3.90	3.83	64	18.18	18.11	14.28	14.28	14.28

As found previously for 40-mesh corn, this method tends to yield somewhat higher results, which it is believed can be attributed to the presence of traces of volatiles remaining in the extracted corn.

Reversibility: Considerable difficulty was experienced in the application of the reversibility method to coarsely ground corn (10-mesh). As shown in the section on De Bruyn methods, coarsely ground corn tends to reach a false equilibrium when dried under mild conditions, because of the unavailability of the "entrapped" water to the desiccant. As a result, the De Bruyn method on the untreated sample underestimates the moisture content.

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The reversibility studies with 40-mesh corn were carried out without encountering the difficulties mentioned above. Three different studies were conducted, and in each case it was found that the ratio of water to volatile loss (decomposition) was essentially the same; *i.e.*, it was found that when the apparent moisture value of the corn equaled the toluene distillation value, decomposition equivalent

to 0.1% had occurred. We have therefore assumed that this same ratio also holds for 10-mesh corn. Observation of the data in Table IV shows that the drying of 10-mesh corn for 16 hours *in vacuo* at 100°C resulted in an apparent moisture value of 14.00%, a value within 0.19% of that obtained by toluene distillation. In view of the work carried out with 40-mesh corn, it has therefore been assumed that a volatile loss equal to 0.1% had occurred during this drying period.

Corn samples dried for varying intervals *in vacuo* at 100°C were redried in a stream of nitrogen at 85°C. Drying was continued for 168 hours, and essentially the same result was obtained for the final three weighings. Typical results obtained after 96 hours of drying are given in Table IV.

TABLE IV

REVERSIBILITY OF 10-MESH CORN

Sample	A Apparent moisture	B Reversible moisture	C Adsorption A–B	D Decomposition B—13.98%	E Calculated true moisture A–D
	%	%	%	%	%
Check	13.76	13.76[1]	—	—	—
Calculated check	13.98	13.98	—	—	—
Vacuum oven, *hrs*					
16	14.00	14.08	—	0.10[2]	—
40	14.40	14.24	0.16	0.26	—
64	14.58	14.34	0.24	0.36	14.22
88	14.65	14.38	0.27	0.40	14.25
136	14.65	14.41	0.24	0.43	14.22
					Av 14.23

[1] Check value too low—false equilibrium.
[2] Decomposition equal to 0.1% was assumed in the light of studies carried out with 40-mesh corn

It will be observed that the moisture value obtained by the reversibility method is in close agreement with the moisture results obtained by the distillation and ether extraction methods. These results will again be referred to in relation to the other data. A second reversibility study was conducted in which the samples were all "wetted" with 50% alcohol prior to drying under the reversibility conditions, but nevertheless it was still found that the check sample yielded low results. Using the assumption made above, it was found that the average calculated moisture value for this second study was 14.18%.

Discussion of the results obtained with 10-mesh corn: The complete data obtained with 10-mesh corn is summarized in Table V. As can be observed from the data given in Table V, the moisture values obtained with the 10-mesh corn range from 13.16% to 14.75%. Values below 14.17% can undoubtedly be rejected as being too low,

TABLE V

SUMMARY OF MOISTURE DATA FOR 10-MESH CORN

Moisture method	Drying conditions	Length of drying	Final moisture value
		hrs	%
Distillation	Benzene—BP 80°C	200	14.17
	Toluene—BP 110°C	140	14.19
Oven drying	Vacuum—80°C	140[1]	14.33
	100°C	125[1]	14.75
	Air—100°C	200[1]	13.16
De Bruyn	Corn alone—50°C	900[1]	13.76
	Corn with alcohol—50°C	900[1]	14.10
Extraction method	Ether extract—75°C—N_2	4.5	—
	Ether-insoluble—100°C—*vacuo*	64	14.28
Reversibility study	Corn predried—100°C—*vacuo*	(1)	14.23
		(2)	14.18

[1] Constancy not obtained.

since the studies conducted with 40-mesh corn clearly indicate that the benzene distillation method causes no decomposition in corn. Similarly, values above 14.28% can be dismissed, since the results by the ether extraction method clearly show that the higher values include the loss of volatiles. The significant moisture range therefore includes values between 14.17% and 14.28%. Three methods have yielded results which fall within this narrow moisture range: the distillation methods (benzene 14.17%, toluene 14.19%), the extraction method (14.28%), and the reversibility method (14.23%, 14.18%).

The results obtained with 10-mesh corn are essentially in agreement with those obtained with 40-mesh corn. The differences noted can be clearly attributed to the effect of granulation. The results demonstrate that the total moisture can be removed from the coarsely ground material if the drying period is sufficiently lengthened to compensate for the inhibiting effect of the coarser granules on moisture loss, and further, that extended times necessary for such moisture removal are justified since these periods produced no decomposition, volatiles, etc. with 40-mesh corn.

The C. I. R. F. Official Reference Moisture Method for Corn

In the studies which have been conducted on 40- and 10-mesh corn, the following methods, both direct and indirect, have narrowed the true moisture content to very narrow limits: (1) distillation with benzene and toluene, (2) De Bruyn, (3) resolution by ether into two fractions, and (4) reversibility.

The distillation procedures stand out as they yield direct moisture and are more adaptable to general laboratory routine. Both benzene and toluene distillation have yielded essentially similar values. Inasmuch as the toluene method is not nearly as time-consuming, the C. I. R. F. adopted the toluene distillation method and a granulation of 20-mesh corn as the official method for the determination of moisture in corn.

The problem of proper sampling and grinding of whole corn in the two studies on 40-mesh and 10-mesh corn was purposely avoided by using, in both cases, a single well-mixed material which, in the case of 40-mesh, can be regarded as homogeneous, and in the case of the 10-mesh, substantially so. To translate these results to laboratory practice requires that the two important factors of sampling and grinding be taken into account. Both can introduce serious error in evaluating the moisture content of corn, independently of the final moisture method used.

As to grinding, it is well known that regardless of the method used, some moisture is lost, particularly if the moisture is high. In the double weighing method, wherein the whole grain is allowed to reach equilibrium in a conditioned room prior to grinding, and the change in weight computed to the final value, there still occurs some moisture loss in grinding.

In order to reduce the grinding loss to a minimum, the C. I. R. F. method proposes that the small Wiley mill be used (No. 4267-P Intermediate Model). This mill permits the quantitative recovery of the sample, and hence the moisture loss during grinding can be determined. It has been found very satisfactory for reduction of whole corn to 20-mesh, with grinding losses which are negligible.

The C. I. R. F. official reference moisture method for corn is as follows: Twenty to 30 g of corn, depending upon the moisture content, is weighed into a tared weighing bottle. The sample is then ground quantitatively in the Wiley mill to 20-mesh and transferred into the weighing bottle, and the moisture loss during grinding is determined. The ground sample is then transferred to a previously air-dried (100°C) distillation flask (250 ml) containing a mat of asbestos, and distillation continued until no water is given off (20 to 80 hours). A more detailed procedure covering apparatus, preparation of the sample and other details is given in the final section following these papers.

Relation of the C. I. R. F. Moisture Method to the Official Methods Now in Common Usage

Having adopted the reference method for the determination of moisture in corn, it seemed desirable to ascertain how the values so

obtained compared to those obtained by state grading laboratories and those within the industry. Such a comparison entailed the preparation of a large quantity of corn of uniform moisture content.

So much has been written regarding the necessity of proper sampling that it is unnecessary to stress again the importance of this factor in moisture studies. Four lots of different grading and moisture were desired. The procedure adopted was as follows: Each lot of corn was allowed to reach equilibrium by being sealed in a metal can for three weeks. It was then transferred to a four-gallon bottle and vigorously shaken for several hours, transferred to one-pound friction-top tins, and stored in a refrigerator. It is believed that this procedure for handling small lots of test samples will lead to more uniformity in whole grain than can be obtained by the use of a Boerner sampler.

This comparative study among laboratories is open to criticism in that it is based on only four samples. However, the results obtained have been in agreement and the data appear to be conclusive. Pound samples were forwarded to (1) two state grain laboratories, (2) eight laboratories within the industry, and (3) samples were retained for moisture tests.

The request to the state grain laboratories was that the moisture be determined by the official method which, in both cases, was the Tag-Heppenstall method. The member laboratories were asked to determine the moisture by methods which they considered most accurate. The samples retained were run by the official C. I. R. F. method and by the official reference method of the U. S. D. A. (100°C electric oven instead of water-jacketed air oven).

The four samples had been graded by the local Merchants Exchange as follows:

Sample No.	Grade	Moisture
1	No. 1 Yellow	11.60%
2	No. 2 Yellow	12.90%
3	No. 2 Yellow	14.40%
4	No. 3 Yellow	16.40%

The moisture contents of the four samples of corn were determined by the C. I. R. F. method with the results shown in Table VI.

In a second study, benzene was used in place of toluene and it was found, as shown in Table VII, that the two distillation methods agree reasonably well. These results also show that the sampling error between the one-pound tins was very small.

The results in Tables VIII, IX, and X show a comparison of the C. I. R. F. method with the now accepted official method of the U. S. D. A. Unfortunately the samples sent to the two government laboratories were considered somewhat small, but those who made

TABLE VI

MOISTURE OF CORN BY THE C. I. R. F. METHOD

Sample No.	Determination	Moisture loss during grinding	Moisture—toluene (60 hrs distillation)	Corrected moisture value for grinding loss
		%	%	%
1	1	0.38	13.31	13.69
	2	0.37	13.24	13.61 Av 13.65
2	1	0.30	13.92	14.22
	2	0.29	13.86	14.15 Av 14.18
3	1	0.44	15.76	16.20
	2	0.45	15.63	16.08 Av 16.14
4	1	0.57	17.00	17.57
	2	0.56	17.03	17.59 Av 17.58

TABLE VII

MOISTURE OF SAMPLE NO. 4 BY BENZENE AND TOLUENE—PERCENT MOISTURE

Benzene (124 hrs distillation)	Toluene
%	%
17.45	17.57
17.50	17.59
Av 17.48	Av 17.58

TABLE VIII

THE C. I. R. F. MOISTURE METHOD FOR CORN VS U. S. D. A. METHOD

Sample No.	C. I. R. F. moisture value	U. S. D. A. 100°C air-oven method (170 hrs) whole corn	U. S. D. A. Tag-Heppenstall values			
			Official moisture value for purchased car	State grain laboratory	Merchants exchange laboratory	Average Tag-Heppenstall value
	%	%	%	%	%	%
1	13.65	11.75	11.60	11.30	11.03	11.31
		11.68 Av 11.72				
2	14.18	12.31	12.90	12.90	12.30	12.70
		12.26 Av 12.28				
3	16.14	14.20	14.40	14.50	14.80	14.56
		14.27 Av 14.24				
4	17.58	15.66	16.40	—	16.45	16.42
		15.64 Av 15.65				

the tests stated that the results obtained represented fairly well the moisture present. The moisture determinations were carried out by the Tag-Heppenstall electric moisture method, which has largely superseded the Brown-Duvel method. The Tag-Heppenstall meter

TABLE IX

C. I. R. F. vs Tag-Heppenstall

Sample	C. I. R. F. method	Tag-Heppenstall	Difference
	%	%	%
1	13.65	11.31	2.34
2	14.18	12.70	1.48
3	16.14	14.56	1.58
4	17.58	16.42	1.16

TABLE X

C. I. R. F. vs Air-Oven—100°C

Sample	C. I. R. F. method	Air-oven—100°C (170 hrs)	Difference
	%	%	%
1	13.65	11.72	1.93
2	14.18	12.28	1.90
3	16.14	14.24	1.90
4	17.58	15.65	1.93

was calibrated, as was the Brown-Duvel, against the water-jacketed oven method.

It will be noted that the Tag-Heppenstall method underestimates the moisture in corn by values ranging from 1.16% to 2.34%. Presumably the lower the moisture of the corn, the greater the difference. Since the Tag-Heppenstall was calibrated against the water-jacketed oven the results given in Table X are even more significant. It was found that the air-oven method at 100°C underestimates the moisture in corn by 1.90% to 1.93%. Since it is generally recognized that the water-jacketed oven method yields lower results than the air-oven method at 100°C, it seems safe, therefore, to conclude that the present official U. S. D. A. method for corn underestimates the moisture content of corn by at least 2.0%.

The results shown in Tables XI and XII clearly indicate that whole corn retains a portion of its moisture with great tenacity.

TABLE XI

C. I. R. F. vs Vacuum Oven—100°C

Sample	C. I. R. F. method	Vacuum oven 100°C (150 hrs)	Difference
	%	%	%
1	13.65	13.00	0.65
2	14.18	13.54	0.64
3	16.14	15.30	0.84
4	17.58	17.08	0.50

TABLE XII

C. I. R. F. vs Air-Oven—130°C

Sample	C. I. R. F. method	Air-oven 130°C (50 hrs)	Difference
	%	%	%
1	13.65	12.74	0.91
2	14.18	13.30	0.88
3	16.14	15.11	1.03
4	17.58	16.68	0.90

Samples of whole corn (10 g) were dried *in vacuo* at 100°C and in air at 130°C for a lengthy period and, as can be observed, a drying period as long as 150 hours *in vacuo* at 100°C is still insufficient for the total removal of the moisture from whole corn. These results show the inadequacy of the present water-jacketed-oven method for the estimation of moisture in whole corn.

Whereas finely ground corn loses its moisture with readiness, whole corn retains a portion of it with remarkable tenacity. During this investigation it has been found that at 100°C *in vacuo*, the total moisture in 40-mesh corn is removable within 4 hours, in 10-mesh corn it required 40 hours, and apparently whole corn requires at least 200 to 300 hours. Granulation plays an important part in rate of water removal. This is again clearly demonstrated in Table XIII.

TABLE XIII

C. I. R. F. vs Company No. 7 Method

Granulation	Company No. 7 method	C. I. R. F. method	Difference
			%
40-mesh	11.56	11.51	+0.05
10-mesh	13.77	14.19	−0.42
¼ grain	12.53	14.18	−1.65

TABLE XIV

Moisture Method of Company No. 7 vs U. S. D. A. Method

Month, 1941	Company No. 7 (monthly average)	State grade moisture (monthly average)	Difference
	%	%	%
January	18.15	17.70	0.45
February	18.18	17.92	0.26
March	17.57	17.37	0.20
April	16.42	15.99	0.43
May	14.69	14.14	0.55
June	13.96	13.63	0.33
			Av 0.36

The method of Company No. 7 is an empirical routine procedure standardized against the water-oven method. It is used as a check against car moisture grading and for factory dry substance purposes. It consists of heating 25 g of ¼ grain corn in a vacuum oven (28½-inch vacuum) at 115°C for 5 hours. The method, in use for years, usually checks car grading and is currently giving values shown in Table XIV.

Relation of the C. I. R. F. Moisture Method to the Methods Now in Use in the Corn Products Industry

As stated in Paper No. I, the corn products industry annually purchases upwards of 100,000,000 bushels of corn, or approximately one-third of the cash corn. This work on moisture in corn has been sponsored by the Corn Industries Research Foundation, because many laboratories of the members believed that official methods do not represent true moisture. Some laboratories have procedures which they believe give more accurate data, while some have adhered to routine methods based on U. S. D. A. official methods. Only one company employs the Brown-Duvel method (No. 8). The others employ oven methods. A comparison of the industry's methods with the C. I. R. F. method is shown in Figure 2 and given in Table XV.

It is of interest to note that the Brown-Duvel method (No. 8) underestimates the moisture in corn samples by values ranging from

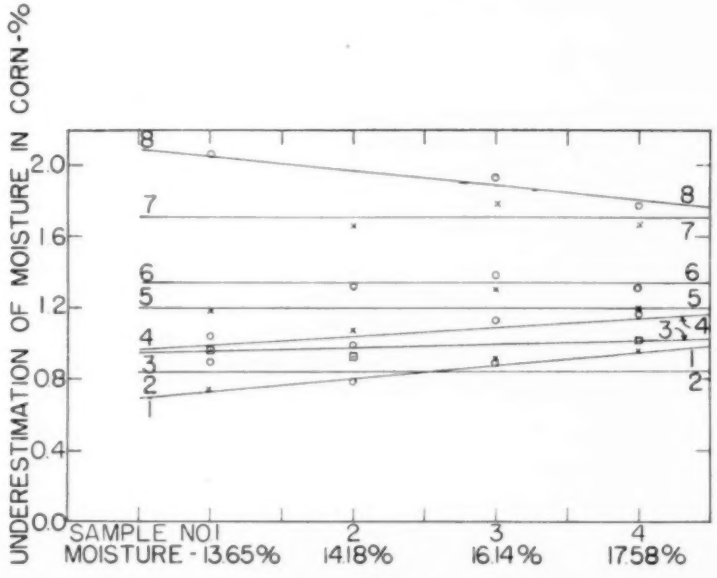

Fig. 2. Relation of the Corn Industries Research Foundation reference method to methods used in the corn milling industry. The numbers refer to companies (see text).

TABLE XV

C. I. R. F. vs Industry Methods

Company	Underestimation of moisture				Average underestimation of companies
	Sample No.				
	1	2	3	4	
	%	%	%	%	%
1	0.74	0.78	0.92	0.97	0.85
2	0.90	0.79	0.90	—	0.86
3	0.98	0.92	—	1.03	0.98
4	1.05	0.99	1.14	1.17	1.09
5	1.18	1.07	1.31	1.22	1.20
6	—	1.32	1.39	1.31	1.34
7	—	1.65	1.79	1.66	1.70
8	2.06	1.38	1.94	1.78	1.79

1.38% to 2.06%, the average difference being 1.79%. These results are in essential agreement with those found previously where the Tag-Heppenstall values were on the average too low by 1.75%, and the air-oven underestimated the moisture by 1.92%.

It will be noted in Table XIV that the method of Company No. 7, over a six-month period, yielded an average moisture value which was 0.36% higher than that obtained by the State Grain Tag-Heppenstall method. This company, as shown in Table XV, underestimated the moisture in corn as compared to the C. I. R. F. method by 1.70%. Indirectly, therefore, we obtain added confirmation indicating that the now accepted official moisture method for corn is, on the average, low by approximately 2.0%.

Conclusions and Summary

The Corn Industries Research Foundation has presented a reference method for the determination of the true moisture in corn. A comparison of this method with the prevailing official methods indicates that:

1. The official water-jacketed-oven method underestimates the moisture in corn by approximately 2.0%, which is a constant difference over the moisture range of 12%–18%.

2. The official Brown-Duvel method underestimates the moisture in corn by values ranging from 1.4% to 2.1%.

3. The official Tag-Heppenstall meter underestimates the moisture in corn by values ranging from 1.2% to 2.3%.

4. The various methods in use by the corn products industry, designed to obtain more accurate moisture data, still underestimate the moisture by 0.8% to 1.8%.

THE DETERMINATION OF MOISTURE IN THE WET MILLING INDUSTRY. III. THE MILL PRODUCTS

L. Sair[1] and W. R. Fetzer[2]

The first two papers in this series covered the determination of moisture in corn. Subsequent papers deal with the determination of moisture in the by-product feeds—gluten meal, corn oil meal, and gluten feed. Inasmuch as chemical as well as physical changes occur during wet milling, which may profoundly alter the nature of the resulting feeds, particularly with respect to their moisture determination, a specific study of each feed product was considered desirable. While standard moisture methods can be applied to a large number of allied products, whether or not true moisture is obtained for the specific product must be determined by a special moisture study of that product. The common assumption that a moisture method can be applied at random is basically one of the major reasons why moisture values have been termed empirical.

The three by-product feeds produced by the wet milling industry are corn gluten feed, corn gluten meal, and corn oil meal. Although the sales specifications among companies may differ to some extent, the following guaranteed analysis may be considered typical:

	Gluten meal	Gluten feed	Corn oil meal
	%	%	%
Crude protein, not less than	41	25	20
Crude fat, not less than	1	2	8
Crude fiber, not more than	5	8.5	10
NFE, not less than	43	43	—

Two of the above feeds, gluten feed and gluten meal, are blends of end products in the miling process. Corn oil meal is simply ground expeller cake. Gluten feed is a blend of coarse hull or bran, steep water, and sufficient corn gluten to make minimum protein guarantees. Gluten meal is a blend of corn gluten, fine fiber or No. 17 tailings, and, at times, some steep water.

The determination of moisture is carried out in the factory not only on the components which make up the feeds but also on the finished feeds. The moisture methods on the latter become important inasmuch as this determination is also made by outside laboratories, making common methods essential. For this purpose relative methods would suffice, but for the purpose of computing yields true moisture values are imperative.

A study of the feed components, which are the hulls, corn gluten, and steep water, was considered desirable in the approach to the

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problem of true moisture of feed by-products, since this investigation would yield an insight into the behavior of the finished feeds. As a result this paper is concerned with a moisture study of the constituents which are used in the manufacture of the finished feedstuffs. It is of importance to note, however, that the behavior of the feeds cannot be altogether predicted from a study of their constituent parts since there is an important point of difference. The component parts carry with them to the driers water containing corn solubles and miscellaneous products of fermentation and enzymic action. During drying, some of these products are lost while others may have reacted to form more stable products. Severe case-hardening, with the resultant entrapment of water, may also occur. As a result the finished feeds may be expected to differ in some respects, both chemically and physically. Before discussing the moisture work on the components, a brief summary of how they originate in the process is in order.

In the wet milling process the water used for washing the final starch moves countercurrently to the corn through a series of separators, in which starch is washed free. The water accumulates solubles and other products of fermentation and enzymic action during this course until it meets the incoming corn. At this stage SO_2 is added to the water in the magnitude of 0.2% to 0.3% and to this the name of steep acid is applied. This water is then run on the corn and recirculated at a temperature of 125°F for 36 to 48 hours to remove corn solubles from the corn berry. At the completion of this steeping process, the water is drawn off for concentration. The Baumé varies from 4° to 6° according to the specific steeping system in use. Concentration is made to 25° Bé or better and the finished product, now known as *steep water*, is ready for blending into feed.

The corn from the steeps passes through a Foos mill, which shreds the berry, leaving the germ relatively intact. The germ is then floated off, washed, dried, and pressed for its oil. The cake when ground is sold as *corn oil meal*.

The hull, fiber, corn gluten, and starch remaining contain a large quantity of grits. In order to separate the starch from the grits, the material passes to a Buhr mill. From the Buhr mill the stream passes through a series of coarse copper reels which separate and wash the *hull*, or *bran*. This material is expressed and dried. It is then ready as a component for compounding into gluten feed.

The effluent now contains starch, corn gluten, and fine fiber. The stream passes a series of reels, made of bolting silk, wherein the fine fiber is separated, washed, and collected wet for blending. It is

known as *fine fiber* or *No. 17 tailings*, the latter name being derived from the silk used.

The effluent now contains starch and corn gluten. The stream passes to the tables where starch is removed by sedimentation and the gluten passed to settling tanks. After a period of settling, the supernatant liquid is decanted and the heavy gluten slurry passed to Merco centrifugals which remove a further quantity of starch which has escaped the tabling operation. The *corn gluten* is then resettled and pressed, usually as a mixture with the fine fiber, which reduces the protein content to the minimum requirements for gluten meal.

The amounts of the end products vary with the company. An approximate breakdown of a bushel of corn is as follows:

	Lbs
Water	8.4
Starch	33.0
Oil	1.5
Corn oil meal	1.8
Hull or bran	2.0
Fine fiber	2.3
Gluten	3.5
Steep water solids	3.5
	56.0

The composition of these products expressed on a dry-substance basis is approximately as follows:

	Hull	Fine fiber	Gluten	Corn oil meal	Steep water solids
	%	%	%	%	%
Crude protein	7.1	14.7	67.5	25.7	45
Crude fat	2.3	1.4	5.7	12.7	—
Crude fiber	19.4	8.5	2.2	9.8	—
Ash	0.8	1.0	2.0	2.9	18
Starch	6.2	49.1	15.0	14.2	—
Pentosans	64.2	25.3	7.6	34.7	—
Gums and sugars[1]	—	—	—	—	37

[1] By difference.

This paper is divided into three sections covering the moisture content of corn hulls, steep water, and corn gluten—the three products which are used in the compounding of the commercial feeds.

Determination of Moisture in Hull and Fine Fiber

Basically, hulls and fine fiber are the same except for degree of subdivision. As a result, a moisture study was conducted solely with the hulls. The methods for moisture analysis were similar to those described in previous papers. The complete data are given in Table I.

TABLE I

THE MOISTURE OF CORN HULLS

Moisture method	Temperature	Length of drying showing period of essential constancy	Moisture range during period of essential constancy	Final moisture value
	°C	*hrs*	%	%
De Bruyn	38	70–114	6.70–6.77	6.77
	60	70–114	6.74–6.78	6.78
	93	70–114	6.79–6.77	6.79
Distillation	Benzene, bp 80°C	5– 7	6.80–6.82	6.82
	Toluene, bp 110°C	5– 7	6.78–6.80	6.80
Vacuum oven	80	16– 45	6.80–6.83	6.83
	110	16– 45	6.77–6.79	6.79

The moisture data for hulls show that the material is stable to drying and that the water is removed rapidly and completely. There can be no doubt that the true moisture is obtained since the various diverse procedures yield results in agreement. These results demonstrate that in the compounded feedstuffs, the hull or fine tailings fraction can be considered as a stable ingredient in which the true moisture can be readily determined.

Determination of Moisture in Steep Water

Practical experience of laboratories in the industry has indicated that steep water is very unstable. The instability of gluten feed during storage, which will be dealt with in a subsequent paper, has been attributed to the steep-water fraction. The nitrogen in steep water calculated as protein (factor 6.25) approximates 50% of the total solids. Inasmuch as the nitrogen fraction is largely composed of hydrolytic products including amino acids, the percentage of nitrogen compounds is probably greater than indicated by the use of the factor 6.25. Steep water also contains a high percentage of ash (15%–20%), some of which is combined with phytic acid. The remainder is largely reducing sugars.

A cold-water extract (0°C) of corn removes approximately 3.9% (DS basis) of soluble material from the corn berry. This is equivalent to approximately 1.9 pounds per bushel. Actual milling practice results in two to three times this amount. This increase is largely in the crude protein fraction as a result of acid hydrolysis in the steeps, together with fermentation and enzymic action in the process. A large percentage of the crude protein fraction is amino acids, which in combination with the sugars represent a product which may be expected to be unstable to drying.

All determinations of steep-water solids by the industry have been considered as approximate, since it is well recognized that the value obtained was dependent on the method of drying. The methods used in the determination of true solids and true moisture of this product will therefore be given in detail, as an example of the method of attack used in the determination of moisture on extremely thermolabile products such as steep water.

Preparation of sample: A composite sample of all heavy steep water produced over a two-day period was made. The pH of the material was 4.32 as determined by glass electrode. The Baumé was approximately 25°. Since steep water of this density carries suspended material, every effort was made to insure uniformity in sampling for the various tests. All weighings for the various determinations were made on the same day.

De Bruyn method: The De Bruyn method was originally applied by Cleland and Fetzer (1942) to the determination of moisture, at low temperatures and high vacuum, in corn sirup, which was then thought to be a heat-sensitive material. Filter Aid was introduced with the viscous material in order to disperse the sample over a very thin layer, from which moisture could be removed readily and completely. Steep water is an analogous material in that, on drying, the mass becomes viscous and gluey, from which moisture is removed with difficulty. Although the De Bruyn determination was designed for low temperatures, the tests on steep water covered a wide range of temperature to permit observation of changes that occurred in the sample during drying. Drying was conducted at 40°, 50°, 60°, 80°, and 95°C.

About 30 g of Filter Aid was run into a De Bruyn flask, test tube inserted, and the same dried in a vacuum oven at 100°C for five hours. The flask was then capped, cooled in a desiccator, and weighed in a tared flask. To the weighed flask was added approximately 20 g of the steep water and the flask reweighed for sample weight. The steep water was then dispersed in the Filter Aid by means of a glass rod which fitted snugly into the test tube. The flask was then attached to the connecting arm of the De Bruyn, an empty flask being placed on the other end and then attached through a large calcium chloride trap to a vacuum pump. After the system had remained overnight at room temperature, the mass of steep water and Filter Aid was slightly damp. At this time the flask was removed and the damp mass worked completely into a uniform, powdered mass. It was then reconnected to the connecting arm and approximately 30 g of P_2O_5 was introduced into the other flask. The De Bruyn was then evacuated by a vacuum pump (Megavac) and the flask containing

the sample was placed in an insulated box arranged with electric lights to give the temperature desired, while the other flask containing P_2O_5 was placed in a bath of running cold water. Cello-Grease (Fisher Scientific Co.) was used for sealing the joints. At the end of 24 hours the vacuum was released by dry air passing through a train containing Drierite and P_2O_5, and the flask containing the sample weighed. The flask was reattached to the connecting arm and a new flask with fresh P_2O_5 attached to the other end. The De Bruyn was again evacuated to 0.1 mm or less and allowed to run for a two- or three-day period before it was again reweighed. Both flasks were rotated daily in order to expose fresh surface. The P_2O_5 flask was renewed as needed. *The surface must remain free-flowing.* Drying was continued until constancy in weight was obtained.

Vacuum oven methods: The following pieces of apparatus were used:

Flasks—250-ml Erlenmeyer standard taper 40/50. These flasks are used interchangeably for oven work and as distillation flasks for distillation work described in previous papers.
Stoppers—Pyrex standard taper 40/12.
Test tubes—Pyrex 100 × 13 mm. Used as pestles for reducing Filter Aid sample mass to a powder. These are weighed with the flask and Filter Aid, and are used as a pestle by the insertion of a glass rod.

Twenty grams of steep water was added to a previously vacuum dried flask containing Filter Aid. The sample was dispersed in the Filter Aid by means of the pestle. It was then given a period of drying in the vacuum oven, after which the sample was reworked to a fine powder. The temperatures employed were 60°, 70°, 80°, and 90°C.

Distillation methods: Apparatus for the distillation methods was similar to that described in previous papers for corn. Approximately 8 g of steep water was run into a weighed flask containing Filter Aid and pestle, which had been previously vacuum dried at 100°C. The flask was reweighed in a tared flask. The sample was quickly incorporated with Filter Aid, 75 ml of solvent was added, and the sample was reworked. The flask was attached to the trap and condenser, the trap filled with solvent, and distillation started slowly.

De Bruyn results: The apparent moisture values obtained by drying steep water in De Bruyns at varying temperatures is shown in Figure 1. After 350 hours of drying, the comparative moisture values obtained were as shown in Table II.

The drying of steep water for 350 hours at 80°C resulted in a loss of dry substance equivalent to 8.9%. Drying at 95°C resulted in a loss of 20.5%. At the lower temperatures, decomposition occurred much more slowly. The De Bruyn at 50°C continued to show a gradual weight change over the total drying period of 900 hours.

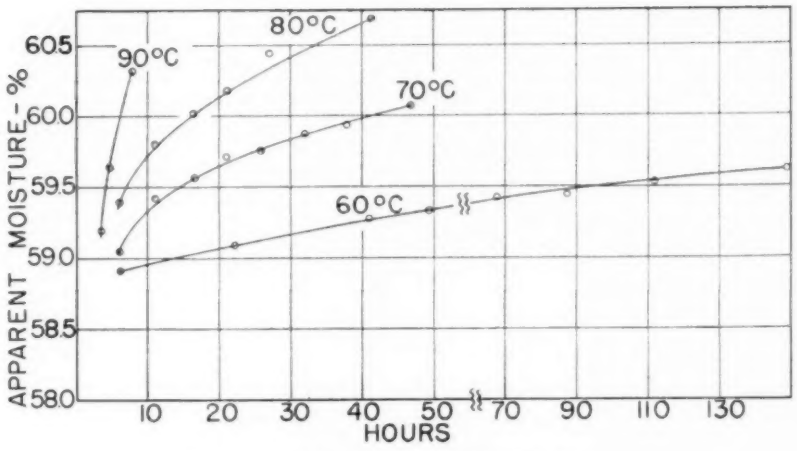

Fig. 1. The drying of steep water in the De Bruyns.

TABLE II

THE DRYING OF STEEP WATER IN DE BRUYNS

Temperature of drying	Apparent moisture	DS decomposition[1]
°C	%	%
40	59.3	0.00
50	59.4	0.00
60	60.4	2.2
80	63.1	8.9
95	67.8	20.5

[1] The value 59.5% was taken as a measure of the true moisture content of this sample of steep water in the light of subsequent work to follow.

At 40°C, constancy in weight was reached. Specific observations on the flasks at various temperatures are as follows:

Drying at 80°C and 95°C: Large quantities of volatiles condensed on the walls of the De Bruyns, particularly at the higher temperature. The Filter Aid steep water mixture became brown to black. At the 95°C temperature, the volatile oil partially crystallized, some fine white needles being formed.

Drying at 60°C: No sign of volatiles noted. The Filter Aid became yellowish within the 90-hour drying period, after which the color slowly increased to light brown (250 hours). The phosphorus pentoxide remained colorless.

Drying at 38°C and 50°C: At the end of the 200-hour drying period, a faint yellow tinge was present at the 50°C temperature, which very slowly increased in hue, and after 900 hours of drying the Filter Aid became yellow.

Vacuum-oven results: The vacuum-oven data for steep water are shown in Figure 2. The samples were removed from the oven, cooled, and weighed at the periods indicated in the graph. As can be observed, the data obtained at the higher temperatures—70°, 80°, and 90°C—compare closely with those obtained in the De Bruyns at elevated temperatures. The continued marked weight loss with

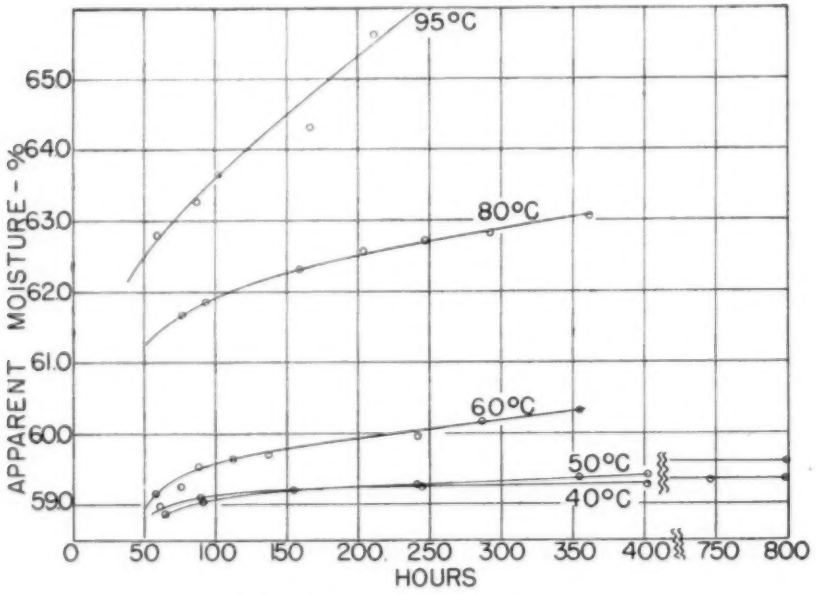

Fig. 2. Vacuum-oven drying of steep water.

drying indicates decomposition. At 60°C, the loss in weight for the first 60 hours was quite rapid, after which the samples continued to lose weight at a very slow rate (0.08% per 44-hour interval). The uniformity in loss in weight suggested that a slow decomposition was in progress.

Distillation results: The distillation data for steep water are given in Table III. With benzene, the water obtained reached a constant value within 8 hours, whereas with the benzene-toluene mixture and toluene, constancy was not reached after 18 hours of distillation. An examination of the flasks after distillation showed only slight color

TABLE III

DETERMINATION OF MOISTURE IN STEEP WATER BY DISTILLATION METHODS

Length of distillation	Solvents		
	Benzene bp 80°C	Benzene-Toluene mixture, bp 92°C	Toluene, bp 111°C
hrs	%	%	%
1	45.80	55.19	53.67
2.25	57.10	60.96	59.63
3.00	58.42	61.09	60.97
6.00	58.55	61.46	61.27
8.00	59.48	61.59	61.42
16.00	59.48	61.84	62.18

formation with benzene, while the mixture of Filter Aid and steep-water solids for the other two solvents had become very dark brown, indicative of decomposition. As will be shown in the next section, the results indicate that the use of benzene is satisfactory for the estimation of the true moisture in a product as unstable as steep water. Toluene distillation, therefore, resulted in an increase of 2.70% of apparent moisture, which on the basis of dry solids is equivalent to the formation of 6.7% of water of decomposition.

Discussion of the moisture methods for steep water: Of all the conditions used, only four methods yield values in which the question of decomposition is limited to a minimum. These values are summarized in Table IV.

TABLE IV

MOISTURE METHODS FOR STEEP WATER

Method	Length of drying	Moisture content	
	hrs	%	
De Bruyn			
40°C	950 (constancy)	59.38	
50°C	357	59.38	
	447	59.47	
	677	59.61	
	900	59.65	Av 59.53
Vacuum oven			
60°C	78	59.42	
	98	59.43	
	122	59.52	
	170	59.61	Av 59.50
Benzene distillation	16 (constancy)	59.48	

In the case of the De Bruyn at 50°C and the vacuum-oven method at 60°C, no constancy in weight was obtainable within a reasonable time interval, and the material was slowly coloring (yellow), which is an indication of decomposition. The moisture values over a lengthy drying period were, therefore, averaged as an estimate of the moisture content. This procedure cannot result in very large error, since the total weight loss during the final 100-hour drying period at 60°C in vacuum was only 0.19%, and in the De Bruyns the change occurring during the drying period from 357 hours to 900 hours was 0.27%.

The two methods yielded constant results, and it is of interest to note that the results are in agreement by 0.1%. The De Bruyn method of drying steep water at 40°C yielded a value agreeing within 0.1% of that obtained by the direct determination of water by benzene distillation. The distillation method also agrees with the De Bruyns at 50°C (0.05%) if the moisture values obtained for the 357- and 900-hour periods are averaged, and also with the vacuum-oven results

at 60°C. The benzene distillation method has been adopted as the official reference method for the determination of water in steep water by the Technical Advisory Committee of the Corn Industries Research Foundation.[3]

Determination of Moisture in Corn Gluten

A composite sample of corn gluten leaving the Merco was filtered and washed exhaustively with water to free the material from the unstable gluten water and solubles. The material was then air dried and ground to pass a 20-mesh sieve. Moisture studies were confined to distillation methods, oven methods, and a single reversibility study. These limited tests were deemed sufficient, inasmuch as a more complete study was under way with gluten meal (corn gluten and fine fiber). The paper on gluten meal follows next in this series. The moisture data are given in Table V.

TABLE V

THE MOISTURE OF CORN GLUTEN

Moisture method	Temperature	Final period of drying	Moisture range during final drying period	Final moisture value
	°C	*hrs*	%	%
Distillation	80			
	Benzene	42–65	8.58	8.58
	110			
	Toluene	42–65	8.66	8.66
Vacuum oven	80	16–45	8.58– 8.67	8.67
	110	16–45	10.06–10.50	10.50

The final constant distillation values for benzene and toluene were 8.58% and 8.66%, a difference of 0.08%. As pointed out in the previous papers, the now accepted A. O. A. C. (1940) official method for the determination of moisture in feeds by toluene distillation states that the distillation is usually over within one hour. At that period, the obtained moisture value for the corn gluten was 8.20%, which is 0.46% lower than that found with continued distillation. The present procedure of limiting the distillation to one hour undoubtedly results in low moisture values.

The vacuum-oven results at 80° and 110°C show considerable disparity. In neither case was constancy in weight obtained, and the apparent moisture values differed by 1.83%. In a second study, the corn gluten was dried *in vacuo* at 100°C, and a continued weight

[3] The above studies were conducted with steep water concentrated directly after removal from the steeping tanks. If the steep water is allowed to undergo partial fermentation prior to concentration, the development of volatile acids may require their partial neutralization before distillation with benzene.

loss with drying was obtained with values intermediate between those previously found. These results again show, as was found for corn, that it is just a question of chance whether oven results at two different temperatures will or will not agree.

The corn gluten dried *in vacuo* at 80°C yielded an apparent moisture value which was 1.83% lower than that obtained by drying the material *in vacuo* at 110°C. Whether or not this marked difference at the elevated temperatures is due to moisture loss or to the loss of volatiles or products of decomposition cannot be determined by the oven data. The toluene distillation method yielded a moisture value of 8.66%, which agrees with the 80°C vacuum value after a drying period of 45 hours, and this agreement suggests that the higher vacuum values at 110°C are not caused by water loss but rather by a dry substance loss. Previous work with corn (see previous papers) has shown that vacuum drying of corn at 110°C for 30 hours results in a volatile loss of 0.60%. Inasmuch as corn gluten contains a higher percentage of ether extract than corn (approximately 50% greater) vacuum drying of this product would be expected to yield an even greater dry substance loss. That this is the case is shown by the following reversibility study.

A full discussion of the reversibility method and its use in the determination of the true moisture content of cereals will be dealt with in the next paper of this series, covering the moisture of gluten meal. A brief outline of the principles was given in the first paper of this series, but a full discussion was reserved for the gluten meal moisture study, since the method was originally applied to that product.

The corn gluten samples dried *in vacuo* at 80° and 110°C, along with control untreated samples, were allowed to reach equilibrium in the Weber oven at 80°C. A stream of dry nitrogen was passed through the oven, in order to eliminate humidity fluctuations. Equilibrium was reached within 48 hours, and the results obtained are given in Table VI.

The reversibility data indicate that the vacuum drying of corn gluten at 80°C results in a slow decomposition or volatile loss. This observation was previously noted for corn. At 110°C *in vacuo*, the decomposition is very marked. By correcting for the decomposition, it is found that the calculated true moisture for the 80° and 110°C vacuum results is 8.48% and 8.59%, respectively. In other words, when the apparent moisture content of corn gluten was 8.67% (80°C *vacuo*) this value included 8.48% of water and 0.19% of volatiles. At 110°C, the apparent moisture value of 10.50% included 8.59% of water and 1.91% of volatiles. This latter moisture figure is in agree-

TABLE VI

REVERSIBILITY AND MOISTURE LOSS OF CORN GLUTEN

Moisture method	Temperature	Length of drying	A Apparent moisture	Reversibility study			
				B Reversible moisture value, 80°C, N_2 48 hrs	C Adsorption A–B	D Decomposition[1] B—8.25	E Calculated true moisture A–D
	°C	*hrs*	%	%	%	%	%
Control air oven, N_2	80	40	8.27	8.25	—	—	—
Vacuum oven	80	45	8.67	8.44	0.23	0.19	8.48
Vacuum oven	110	45	10.50	10.16	0.34	1.91	8.59

[1] Includes volatile loss.

ment with the distillation results. The lower moisture value indicates that the 45-hour drying period at 80°C was insufficient for the total removal of the water.

Summary

The moisture content of the three mill products which are used in the compounding of the commercial feedstuffs in the corn industry was determined.

The hulls or fine tailings are stable, and the true moisture content can be determined by a variety of methods which includes distillation with benzene or toluene, drying in a De Bruyn over P_2O_5 at temperatures ranging from 40° to 90°C, and by vacuum drying at 80° or 110°C.

Steep water is an unusually thermolabile product, which undergoes decomposition at low temperatures (60°C). The moisture cannot be removed directly from this product by oven or distillation methods, since on drying it becomes extremely viscous and severe case-hardening occurs. It is necessary first to disperse the material on Filter Aid. The material can then be dried to constancy by two moisture methods, one direct and the other indirect, which are in agreement. The values obtained by drying the material to constancy in De Bruyns at 40°C agree with the direct determination of moisture by benzene distillation. Toluene distillation of steep water yields higher moisture values, equivalent to 6.7% of water of decomposition. The benzene distillation method has been accepted as the official reference method for the determination of the true solids in steep water by the Technical Advisory Committee of the Corn Industries Research Foundation.

The true moisture in corn gluten cannot be satisfactorily determined by oven methods. Vacuum drying of this product results in a

marked loss of volatiles (1.91% at 110°C). The toluene distillation method is satisfactory for the determination of moisture in corn gluten.

Literature Cited

Association of Official Agricultural Chemists
1940 Official and tentative methods of analysis of the Association of Official Agricultural Chemists (5th ed.).
Cleland, J. E., and Fetzer, W. R.
1941–42 Determination of moistures in sugar products. II. Corn products. Ind. Eng. Chem. (Anal. Ed.) **13**: 855–860 (1941); **14**: 27–30; 124–129 (1942).

THE DETERMINATION OF MOISTURE IN THE WET MILLING INDUSTRY. IV. CORN GLUTEN MEAL

L. SAIR[1] and W. R. FETZER[2]

This paper is a continuation of a series dealing with the moisture in corn and its feed by-products. The first two papers dealt with corn, while the third dealt with the moisture study of the blending components—namely, the hulls, corn gluten, and steep water, which make up the commercial by-product feeds. It was thought that a study of these constituents would lead to a better understanding of the moisture determination in the compounded feedstuffs. Three commercial feed products produced by the corn industry are sold under the trade names of corn gluten meal, corn gluten feed, and corn oil meal. This paper deals with a moisture study of gluten meal.

In the wet milling process, the corn gluten is separated from the starch by tabling, the fraction floating off being termed corn gluten. The slurry is then passed to a centrifugal which reduces the starch content to approximately 20%. In the wet stage, this material is mixed with tailings (fine fiber) and is run through either a filter press or string filter, after which it is kiln dried. The dried product is gluten meal. A typical analysis follows:

	%
Moisture	7.6
Crude protein	43.8
Crude fat	0.70
Ash	1.2
Fiber	3.8
NFE (by difference)	43.6

Studies were made on composite samples of corn gluten meal produced by two members of the industry. The methods of moisture approach were essentially similar to those previously described for corn, which included distillation methods, oven methods, the De Bruyn

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method of drying in high vacuum over P_2O_5, and the reversibility method. Both samples were ground to pass a 20-mesh sieve.

In all cases, the difference between duplicates includes the sampling error, since the duplicate samples for each determination were taken from different bottles of gluten meal. The individual values for all determinations with the exception of the oven results are included in this paper. The difference between the duplicate values for the oven determinations rarely exceeded 0.03%, the SE from the means for the duplicates being 0.02%.

Moisture Content of Gluten Meal No. 1

The moisture results obtained by the use of distillation methods, De Bruyn methods, and by oven methods for the first sample of gluten meal (No. 1) tested are given in Table I. It should be made clear

TABLE I

THE MOISTURE OF GLUTEN MEAL NO. 1

Moisture method	Temperature	Length of drying, showing period of essential constancy	Apparent moisture		
			Moisture range during period of essential constancy	Final moisture value	
				Duplicates	Average
	°C	*hrs*	%	%	%
De Bruyn	40	240–340	7.80–7.84	7.84	7.84
	60	240–340	7.81–7.83	7.85–7.81	7.83
	90	80–200	7.89–7.92	7.90–7.94	7.92
Oven drying	*Vacuo* 80	60–160	7.86–7.88	7.89–7.88	7.88
	90	16– 60	7.88–7.94	7.94–7.95	7.94
	100	(1)24– 80	7.86–7.90	7.88–7.92	7.90
		(2)16– 60	7.89–7.90	7.91–7.89	7.90
	120	40– 90	8.12–8.16	8.16–8.17	8.16
	Air 100	5–160	7.42–7.38	7.36–7.39	7.38
	130	24– 60	7.94–8.01	8.03–7.99	8.01
Distillation	Benzene	30– 48	7.76	7.69–7.76–7.75 7.82	7.76
	Toluene	30– 48	7.87	7.78–7.84–7.92 7.94	7.87

that for each moisture procedure, the test was continued until an essential equilibrium value was obtained; that is, for each condition of temperature, pressure, desiccant or solvent, drying was continued until a constant value characteristic of the apparent moisture resulted.

The data given in Table I show that the gluten meal sample was markedly stable to heat, which is surprising in view of the results previously obtained for corn gluten (see paper No. II). Vacuum drying of corn gluten at 80° and 110°C resulted in an apparent moisture

difference of 1.8%. This is attributed, in view of the similar moisture values obtained by benzene and toluene distillation, and also reversibility studies, to volatile loss. Corn gluten contains 5.5% to 7.0% of fat. With gluten meal, this marked difference in oven data does not occur, thus indicating a loss or fixation of the volatiles during kiln drying. Analytical data indicate that some change does occur during drying, since the ether extract values for corn gluten and gluten meal approximated respectively 6.0% and 1.0%. The dilution of corn gluten with fine tailings in the preparation of gluten meal amounts to approximately 40%, which in terms of ether extract should reduce the fat value in the gluten meal to 3.6%. This discrepancy in fat content of the gluten meal is attributable largely to a fixation within the protein meshwork and because of case-hardening during kiln drying. If gluten meal is first subjected to a mild acid treatment prior to ether extraction, a higher ether extract value is obtained. The effect of drying, therefore, increases the stability of gluten meal with respect to volatile loss during oven drying.

The moisture range obtained by the various moisture methods varied from 7.38% (air oven, 100°C) to 8.16%. The low air-oven values are attributable to the property in gluten meal of retaining water at this temperature, as will be shown in a following section. The significant moisture range can be narrowed to include values between 7.76% and 8.16%, a difference of 0.40%.

The drying of gluten meal in De Bruyns at high vacuum (0.01 mm) over P_2O_5 was conducted at 40°, 60°, and 90°C. It will be noted that a 50°C differential in temperature resulted in only an 0.08% increase in moisture. The obtained moisture range varied from 7.84% to 7.92%.

Vacuum drying was conducted at 80°, 90°, 100°, and 120°C. The final moisture values obtained for the 80°, 90°, and 100°C results were within very narrow limits, a difference of only 0.06% being obtained. The values ranged from 7.88% to 7.94%, which are in essential agreement with those obtained with the De Bruyns. At the 120°C temperature, the obtained moisture value shows an abrupt increase equivalent to 0.26%. Inasmuch as the 80° and 100°C values were essentially similar, this increase at 120°C suggested decomposition. That this was the case will be shown later in the section of this paper dealing with reversibility.

Distillation with benzene and toluene yielded results which differed by 0.11%. This may be explained in the method of drying of this product. The material, by impact, often dries to a hard horny mass. Even with the material ground to 20-mesh, it might be expected that the low boiling solvent (benzene 80°C) might find it difficult to remove

all the water. The values obtained by toluene distillation (7.87%), however, agree with the vacuum oven results at 80°, 90°, and 100°C and with the De Bruyn values at 90°C.

The results, as given in Table I, indicate that the moisture values fall roughly into three groupings—a lower moisture range of 7.76% to 7.85%, an intermediate group varying from 7.87% to 7.94%, and higher values extending from 8.01% to 8.16%. The lower results were obtained by subjecting the gluten meal to mild conditions, the feed being dried in De Bruyns at 40° and 60°C or distilled with benzene. The higher moisture values were obtained by subjecting the gluten meal to a relatively drastic treatment. In one case, the feed was dried for 60 hours in an air oven at 130°C. A second procedure involved vacuum drying for 90 hours at 120°C. The intermediate moisture values include the De Bruyn drying at 90°C, vacuum drying at 80°, 90°, or 100°C, and toluene distillation.

Studies which will be given in the next section throw considerable light on the problem of determining which of the above moisture groups is representative of the true moisture content of gluten meal.

Moisture loss and reversibility: The principles underlying the application of the reversibility method for the determination of the true moisture content of corn were briefly outlined in the first paper of this series. Since this method was originally used during the investigations conducted with gluten meal, a more lengthy discussion of the principles and uses of the reversibility method will be given here.

Van Bemmelen (1910) in his studies on the moisture relations of silicic acid gels showed that the desorption (moisture loss) and adsorption (moisture gain) curves did not coincide over the whole moisture range. The desorption and adsorption curves coincide for the first increment of water adsorbed, after which the previously dried gel shows a diminishing ability to adsorb moisture with increasing moisture content, as compared to the original gel. This behavior has been termed hysteresis. Urquhart and Williams (1925) have obtained essentially similar results with cotton. They found that the adsorption and desorption curves coincide, when dried cotton (vacuum oven, 110°C) is allowed to adsorb only 1% of water. Beyond this, the adsorption curve shows an increasing variance with the desorption curve with increase in moisture content. The heat-treated cotton loses its ability to hold as much water. Pidgeon and Maass (1930) have reported essentially similar results for cellulose. The two curves coincide for the first moisture uptake, after which hysteresis occurs.

The findings of these investigators offer the basis upon which the reversibility method has been developed for the determination of the true moisture content in cereals. They found that the drying of

silicic acid gels, cotton, and cellulose is completely reversible if the dried materials are allowed to readsorb only 1% of moisture. If during drying the products have undergone some decomposition, or if a volatile has been removed, then this complete reversibility is unobtainable. It was thought that if this behavior could be found applicable to cereals, it would be of value in the determination of moisture because decomposition or the loss of volatiles could then be detected.

Davidson and Shorter (1930) made use of the desorption-adsorption relation in the determination of moisture in cotton. They dried cotton at 90°, 100°, 110°, and 120°C and then redried all the products at 90°C after moistening. Equilibrium values in excess of those obtained by cotton dried directly at 90°C were considered to be attributable to decomposition. In a second study, cotton was dried at temperatures of 100°, 135°, 160°, 182°, 200°, and 212°C. These products were then redried at room temperature over P_2O_5, and the obtained dry-substance value was compared to a control dried directly over P_2O_5. Davidson and Shorter concluded that the true moisture of cotton is obtainable, and pointed out that their results were not in agreement with those obtained by Nelson and Hulett (1920).

The desorption-adsorption method for the determination of moisture was applied to corn (No. I of this series) and the calculated true moisture was found to be in agreement with that obtained by other moisture methods, thus indicating the utility of this procedure.

The data obtained for gluten meal indicate that for each condition of temperature, pressure, and desiccant, the material attained an equilibrium value. For example, in an air oven at 100°C, gluten meal attained constancy during the period from 5 to 140 hours, the moisture value ranging from 7.33% to 7.43%. This apparent moisture value fluctuated from day to day within this narrow range, showing no particular trend. Inasmuch as the 5- and 140-hour period values are in close agreement, these results preclude the possibility of decomposition with a resultant loss in weight. Therefore, it would be expected that this particular sample of unaltered gluten meal should, irrespective of its previous drying treatment, attain a value of 7.40% when allowed to reach equilibrium at 100°C in air. This value, in a sense, can be considered as a *physical* constant. Similarly, it was found that the gluten meal when dried in a vacuum oven at 100°C attained an equilibrium at 7.90%.

The following experiments were carried out which showed that the values obtained in the air and vacuum ovens at 100°C were completely reversible:

1. When the vacuum-dried gluten meal was replaced in the air oven, it adsorbed 0.5% of moisture and reached the apparent moisture value of 7.40%, characteristic of unaltered gluten meal.

2. When air-dried gluten meal was placed in the vacuum oven, it lost sufficient moisture to yield a value of 7.75%. This value is still 0.15% too low as compared to direct vacuum drying of gluten meal. That this difference was due to "case-hardening" in the air oven was proved by moistening the air-dried samples prior to transfer. By first softening the feed, the vacuum oven values were increased to 7.88%, a value similar to that obtained by drying the feed direct.

These results show that gluten meal can adsorb at least 0.5% of moisture reversibly and that in this respect, gluten meal behaved in a manner similar to silicic acid, cotton, and cellulose. Moreover, it follows that the vacuum-oven value at 100°C included no losses due to decomposition or the loss of volatiles.

The gluten-meal samples dried for 60 hours at 130°C in an air oven (8.01%) were treated similarly. In this case, the feed reached an equilibrium value of 7.88%, which is a value far removed from that obtained with unaltered gluten meal (7.40%). These results indicate that the heating of gluten meal at 130°C for a long period caused some physical or chemical changes, thus reducing its water-binding capacity. It was thought that possibly the failure for reversibility might be caused by an unusual case-hardening of the material at the high temperature. Therefore the samples were moistened and replaced in the air oven at 100°C. An apparent moisture value of 7.86% was obtained, thus eliminating the possibility that this factor was responsible for the differences found.

Various other experiments of a similar nature have been carried out, and the data are given in Table II. These data indicate that the moisture-loss curve of this sample of gluten meal is completely reversible if the apparent moisture obtained does not exceed 7.88%. Beyond this, complete reversibility is not obtainable, the reversible value being greater than 7.40% to almost exactly the same amount as the apparent moisture value exceeded 7.88%. In all cases, the samples adsorbed essentially the same amount of water, 0.46%–0.50%. It appears, therefore, that the values greater than 7.88% are due to losses attributable either to the loss of volatiles or to the loss of decomposition products.

By subtracting the percentage of decomposition obtained by the various vacuum-oven treatments from the apparent moisture, it can be noted that the calculated true moisture values fall within a very narrow moisture range, 7.86%–7.90%.

TABLE II

REVERSIBILITY AND MOISTURE LOSS OF GLUTEN MEAL

Moisture method	Temperature	Interval of drying	Reversibility study				
			A	B	C	D	E
			Apparent moisture	Reversible moisture value at equilibrium in air oven at 100°C (72 hrs)	Adsorption of water A–B	Decomposition[1] B—7.40%	Calculated true moisture A–D
	°C	hrs	%	%	%	%	%
Control air oven	100	24	7.38	7.40	—	—	—
Vacuum oven	100	21	7.88	7.39 7.41 Av 7.40	0.48	0.00	7.88
Air oven Vacuum oven	130 100	4 16	7.88	7.43 7.42 Av 7.42	0.46	0.02	7.86
Vacuum oven	90	60	7.94	7.42 7.46 Av 7.44	0.50	0.04	7.90
Vacuum oven	100 120	64 4	8.00	7.52 7.48 Av 7.50	0.50	0.10	7.90
Vacuum oven	100 120	64 90	8.16	7.69 7.68 Av 7.68	0.48	0.28	7.88
Vacuum oven	120	90	8.18	7.68 7.69 Av 7.68	0.50	0.28	7.90 Av 7.89

[1] Decomposition includes the loss of volatiles.

Discussion of the results obtained for gluten meal No. 1: The data given in the foregoing section indicate that the true moisture content of the sample of gluten meal under examination lies within the limits of 7.86% to 7.90%. Gluten meal dried to constancy in a vacuum oven gave the following results:

Temperature of oven °C	Apparent moisture %
80	7.88
90	7.94
100	7.90
120	8.17

The moisture results obtained at 80°, 90°, and 100°C are in essential agreement. At the higher temperature (120°C) a distinct break occurs, resulting in an apparent increase of 0.27% in moisture. It is generally recognized that water is held by colloidal materials with different degrees of tenacity and hence, for each increase in temperature, more moisture is liberated if additional water is present. This was not the case for this sample of gluten meal, and therefore the break must indicate some loss other than water held by purely me-

chanical or physical forces. This might be explained as caused by water loss, but if so this water is held by forces of an order different from the water removed at the lower temperatures.

The De Bruyn method of drying *in vacuo* over P_2O_5 yielded moisture values ranging from 7.83% to 7.92%. The reasons for the slightly lower results obtained at 40° and 60°C (7.83%) are undoubtedly the same as was previously reported for corn ground to only 10-mesh (No. I of this series). The hard grits reduce the availability of the water when dried at low temperatures. As a consequence, a false equilibrium results. This will be shown in more detail in the second study of a gluten meal.

Toluene distillation yielded a moisture value of 7.87%. This method determines water directly and the value obtained is in agreement with the vacuum-oven results (7.88%–7.94%), the De Bruyn method at 90°C (7.92%), and with the reversibility studies (7.89%). The results of this investigation show that the toluene distillation method, when carried to completion, removes the total water from gluten meal.

The Moisture Content of Gluten Meal No. 2

Upon completion of the study on gluten meal No. 1, the work was repeated on a second sample composited by a different company. The object was to determine the variability that might exist from the moisture standpoint in gluten meals produced by different commercial processes.

The moisture content of the gluten meal was determined by vacuum oven drying at 90°, 100°, 110°, and 120°C, by air drying at 100°C, by the toluene distillation method, and by reversibility studies. A summary of the oven and distillation data is given in the following table, and shown in Figure 1.

TABLE III

THE MOISTURE OF GLUTEN MEAL NO. 2

Moisture method	Temperature	Interval of drying	Moisture range	Maximum apparent moisture value
	°C	*hrs*	%	%
Air oven	100	24–96	10.98–11.04	11.04
Vacuum oven	90	16–40	11.20–11.24	11.24
	100	16–72	11.40–11.49	11.49
	110	8–40	11.47–11.64	11.64
	120	2–16	11.41–11.68	11.68
Toluene distillation	bp 110	4–28	11.39	11.40 11.37 11.39 Av 11.39

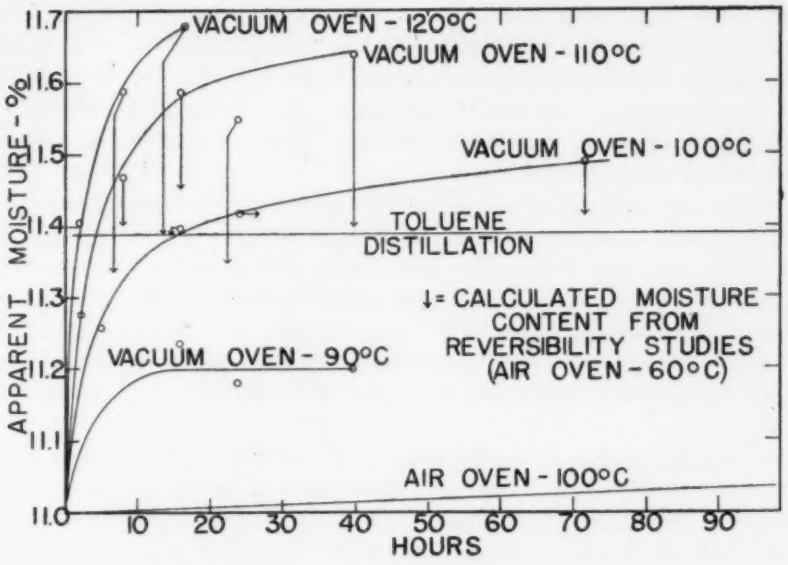

Fig. 1. The determination of moisture in gluten meal, No. 2.

The results obtained with the second sample show a considerable variation from those previously obtained. The first sample was much more stable, the meal exhibiting a constancy in weight when heated *in vacuo* at 100°C for a considerable period. A difference of only 0.30% was obtained between vacuum drying at 80° and 120°C. With the second sample, the difference between 90° and 120°C amounted to 0.44%, even though the material was heated for only 16 hours at the higher temperature in contrast to 90 hours for the first sample. That this variability occurs can be readily understood when it is realized that the gluten water from which the corn gluten settles varies markedly in stability, dependent on the conditions under which it is made. Corn gluten, as it leaves the string filter, contains 60% of gluten water, which, in turn, contains approximately 2% of solubles. The dried gluten, therefore, contains 1.2% of gluten-water solubles. A second variability resides in the method of drying the gluten meal. Previous work (paper No. II) showed that corn gluten contains material which readily volatilizes *in vacuo* at higher temperatures. These volatiles were apparently firmly fixed in gluten meal No. 1 but only partially in the second sample.

The data in Table III indicate a different apparent moisture value for each oven condition. At 90°C *in vacuo*, constancy was reached at a moisture value of 11.24%, the value shifting only 0.04% in 24 hours. At 100°C the moisture value was increased to 11.49%,

the value increasing 0.09% in the last 56 hours of drying. The lower value, as will be shown later, is attributable to severe case-hardening, which may readily occur in kiln drying, with a resultant false equilibrium. At the higher drying temperatures (110°–120°C) the values showed a constant increase. Toluene distillation yielded a constant value of 11.39%.

From the above results it is impossible to make any deductions regarding the true moisture content of the feed sample. It might be surmised that the true value lies in the region of 11.40%–11.49%, since with continued heating for 56 hours *in vacuo* at 100°C, the value shifted only 0.09%. However, this assumption would at best be a compromise, since it might be argued that since constancy was reached *in vacuo* at 90°C, the moisture value of 11.20%–11.24% should be chosen.

The reversibility method has been of particular value in clarifying the oven data. The moisture dishes containing the feeds dried *in vacuo* at 100°, 110°, and 120°C were compared to unheated gluten meal samples at a temperature which precluded decomposition. Inasmuch as it appeared as though a slow decomposition may be

TABLE IV

MOISTURE LOSS AND REVERSIBILITY OF GLUTEN MEAL AT 60°C

Oven treatment	Interval of drying	Reversible experiment				
		A	B	C	D	E
		Apparent moisture	Reversible moisture value, 96 hours	Adsorption A–B	Decomposition B—8.80%	Calculated true moisture A–D
	hrs	%	%	%	%	%
Control		8.70	8.80	—	—	—
Vacuum oven 100°C	5	11.26	8.74	2.52	—	—
	16	11.40	8.81	2.59	0.01	11.39
	24	11.42	8.80	2.62	0.00	11.42
	72	11.49	8.87	2.62	0.07	11.42
Vacuum oven 110°C	2	11.28	8.80	2.48	0.00	—
	8	11.47	8.87	2.60	0.07	11.40
	16	11.58	8.92	2.66	0.12	11.46
	24	11.55	9.00	2.55	0.20	11.35
	40	11.64	9.03	2.61	0.23	11.41
Vacuum oven 120°C	2	11.41	8.90	2.51	0.10	11.31
	8	11.59	9.05	2.54	0.25	11.34
	16	11.68	9.08	2.60	0.28	11.40
						Av 11.39

occurring in the air oven at 100°C, the reversibility study was conducted at a lower temperature (air oven, 60°C). Preliminary studies showed that the desorption and adsorption curves for gluten meal were reversible, exhibiting no hysteresis, if the dried feeds were allowed to adsorb as much as 3.0% of water. It was necessary, however, in order to obtain this reversibility, first to moisten both the dried samples and the check, thus putting them on a comparable basis. The vacuum-dried samples and the controls were, therefore, moistened with 8 ml of water and allowed to reach equilibrium in the air oven at 60°C. Considerable weight fluctuations occur at this temperature because of outside humidity changes, and later studies, as shown for the moisture work with corn and corn gluten (papers I and II), eliminated this factor by the use of a constant-humidity chamber.

The reversibility data are given in Table IV, from which it can be observed that the average calculated true moisture is 11.39%, which is identical with that obtained by toluene distillation. This agreement, particularly in view of the results obtained with the first sample of gluten meal, which was much more stable, leads again to the conclusion that the toluene distillation method is satisfactory for the determination of the true moisture in gluten meal.

Summary

Moisture studies were conducted on composite samples of gluten meal produced by two companies in the wet milling industry. The results indicate that the true moisture content of gluten meal can be determined. It was found that gluten meal produced by different companies shows variability from the stability standpoint, and that a standard oven procedure would be unreliable as a reference method for the determination of the true moisture in this product. Case-hardening and the presence of volatiles affect the oven results. As found previously for corn and corn gluten, the distillation methods are much more reliable for the determination of true moisture. The toluene distillation method, carried to completion, is satisfactory for the determination of the true moisture in gluten meal, and this method has been accepted as the official reference method by the Technical Advisory Committee of the Corn Industries Research Foundation.

The use of the reversibility method for the determination of the true moisture in corn and gluten meal has indicated that this procedure should have a wide application in the determination of the true moisture content in a large variety of products. The method is simple, yields consistent results, and appears to be based on sound premises.

Literature Cited

Davidson, C. F., and Shorter, S. A.
1930 The dry weight of cotton. J. Textile Inst. **21**: T165–T178.
Evans, J. W.
1941 Private communication, unpublished.
Nelson, O., and Hulett, G. A.
1920 The moisture content of cereals. Ind. Eng. Chem. **12**: 40–45.
Pidgeon, L. M., and Maass, O.
1930 The adsorption of water by wood. J. Am. Chem. Soc. **52**: 1053–1069.
Urquhart, A. R., and Williams, A. M.
1925 The moisture relation of cotton. II. The absorption and desorption of water by soda-boiled cotton at 25°C. J. Textile Inst. **15**: T433–T442.
Van Bemmelen, J. M.
1910 Die Absorption. Gesammelte Abhandelungen über Kolloide und Absorption. 196, 214, 232, Dresden, Steinkopff, 548 pages.

THE DETERMINATION OF MOISTURE IN THE WET MILLING INDUSTRY. V. CORN GLUTEN FEED, UNSWEETENED AND SWEETENED

L. Sair[1] and W. R. Fetzer[2]

In supporting the investigations reported in this series of papers the Technical Advisory Committee of the Corn Industries Research Foundation clearly indicated that they were not interested in empirical moisture methods. They stated that the objective would be successfully accomplished only if methods were devised which would determine the true moisture content of each product. Later, if desired, the work could be utilized in a more practical form by the calibration of simpler moisture methods against the reference procedures.

It was recognized at the inception of these studies that all former investigations had led to the conclusion that only empirical moisture methods were possible, since "free water" could not be differentiated from "bound water" and from "water of constitution." This belief suggested that water is held by cereals with varying degrees of tenacity and that a portion of it is so tightly bound that its removal cannot be effected without decomposing the material under test. In view of this situation, the moisture method has been considered as a tool which will yield relative rather than absolute moisture results.

Moisture studies with corn, corn gluten, steep water, hulls, and gluten meal have led to conclusions contrary to those previously held regarding moisture in cereals. The moisture problem for corn and its feed products was approached by several experimental methods not hitherto applied to cereals and it was found that the true moisture content of each product could be determined, and that there is a clear differentiation between free and adsorbed water and water of constitu-

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tion. The results indicated that there is no need to rely on empirical methods for the determination of moisture. Studies with corn (paper No. I) showed that the present official moisture methods underestimate the moisture in corn by approximately 2.0%; in other words, 100 pounds of corn contain 2 pounds less dry substance than formerly thought. These results offer an explanation for the unaccountable loss of 2% to 3%, which in spite of extensive studies could not be accounted for previously in the wet milling industry. Studies with steep water, hulls, and corn gluten (paper No. II) indicated that the present procedures were too empirical. The current methods in use for steep water overestimated the moisture by 2%–4%. Vacuum drying of corn gluten resulted in a marked loss of volatiles. Oven procedures in general yielded only empirical values. Similarly, the practice of limiting the toluene distillation procedure to 1 to 2 hours consistently underestimated the moisture in products such as corn, corn gluten, and gluten meal.

This paper covers moisture studies dealing with gluten, hydrol, and molasses feeds. Corn gluten feed is produced by kiln drying, usually in a two-step process. In the first step, concentrated steep water is added to previously dried hulls which are then passed through a drier at a rate that will produce a product not completely dry. Additional steep water and corn gluten are added to this product, the mass thoroughly blended, and passed to a second drier which reduces the moisture to trade requirements. Thus a moisture test on this product becomes essentially one for steep water plus manipulation to free moisture encased and sealed in the hulls and gluten by the external layer of steep water. Sweetened feeds are produced by mechanically blending hydrol or molasses with finished feed, followed by subsequent processing.

The moisture studies reported in this series were not conducted in the order reported, but rather in what was later considered a more logical sequence. The first studies were carried out with gluten feed. The outcome of this investigation offered the basis upon which the other studies were conducted. In many respects this product offered more problems than the others. Various factors which enter into the moisture test, such as case-hardening, instability, presence of volatiles, and variability of the feeds themselves, play a pronounced role in the determination of moisture in gluten feed. The feed is compounded from both feed products and an unstable sirup (steep water), and as a result the determination of moisture in this product includes all the difficulties attendant on its estimation in an unstable sirup combined with the problem of a feed product. Moreover, in gluten feed the problem is further complicated by the fact that the steep water is

dried upon the hulls in kilns during which time it hardens into a resinous coat, thus entrapping the enclosed water.

The unsuitability of present existing official methods for the determination of moisture in gluten feed can be noted from the results given below. Inasmuch as the feed is composed of both a sirup and a feed, the methods used included those given under grain and stock feeds and also under sugars.

Method—A. O. A. C.	Apparent moisture %
Grain and stock feed:	
Drying over P_2O_5 *in vacuo* at 40°C (800 hrs)[1]	9.09
Vacuum oven—100°C—5 hrs[2]	11.10
Vacuum oven—100°C—24 hrs	12.80
Toluene distillation—1 hr[3]	11.10
Toluene distillation—10 hrs	12.25
Air oven—135°C—1 hr	14.35
Sugar and sugar products:	
Air oven—100°C—10 hrs	11.60
Vacuum oven—80°C—16 hrs[4]	9.76

[1] The official method uses concentrated sulfuric acid in a vacuum desiccator at room temperature.
[2] The official method states that the product should be heated for 5 hrs or longer until constancy is reached.
[3] The official method states that the distillation is usually complete within 1 hour.
[4] The official method states that a temperature of 70°C should be used and that weighings should be made at 1-hr intervals after the first 2-hr period. Constancy was not reached in the above determination.

The discrepancies shown above demonstrate the present empirical status of the moisture determination. They also indicate the undesirability of the inclusion of a large variety of products under one general procedure. Gluten feed is considered as a grain or stock feed. Yet obviously, all the official methods, or possibly none, are suited to the determination of moisture in this product. Grain and stock feeds differ markedly in their properties and behavior. Because of their nature and mode of preparation, the feeds produced in the brewing industry, the milling industry, the distilling industry, the wet milling industry, and others will exhibit differences that must be accounted for in an official moisture procedure. If the above official methods had been applied to gluten meal, the differences found would have been much smaller than with gluten feed. Yet, all these feed products are considered as similar from the moisture standpoint. Corn oil meal is the ground expeller cake obtained from the expression of corn oil from dry corn germs under high pressure. During this process, some moisture is firmly fixed in the meal and can be removed only in a moisture determination by drastic treatment. This procedure will be covered in the next paper of this series. The results of these investigations indicate that the present official methods do not account for the variability that exists in different feed products.

Plan of investigation: The initial plan involved the application of distillation and De Bruyn procedures. Later, in view of the resulting

data, various modifications were introduced which will be dealt with under the appropriate headings. The experimental methods originally applied were identical to those described for corn (paper I). A study was first made on a single composite sample of feed ground to pass a 20-mesh sieve. All samples were placed in 4-ounce screw-top bottles which were stored in the refrigerator until required. Routine analysis of the first sample of gluten feed used is given below:

Moisture	11.19%
Crude protein (N × 6.25)	25.2 %
Ether extract	3.7 %
Crude fiber	7.6 %
Ash	8.6 %
NFE (by difference)	43.7 %
Acidity as hydrochloric acid	1.33%
pH[1]	5.2 %

[1] Ten g of feed in 100 ml of water was allowed to stand for 30 minutes before the determination by glass electrode.

Moisture results: The apparent moisture values obtained by drying the feed at various temperatures in De Bruyns, and also by distillation with benzene, toluene, and xylene are shown in Figures 1 and 2 and in Table I. The feed sample used for this study was similar to that used for the comparative study by the A. O. A. C. methods. The De Bruyn procedure involves the drying of the feed

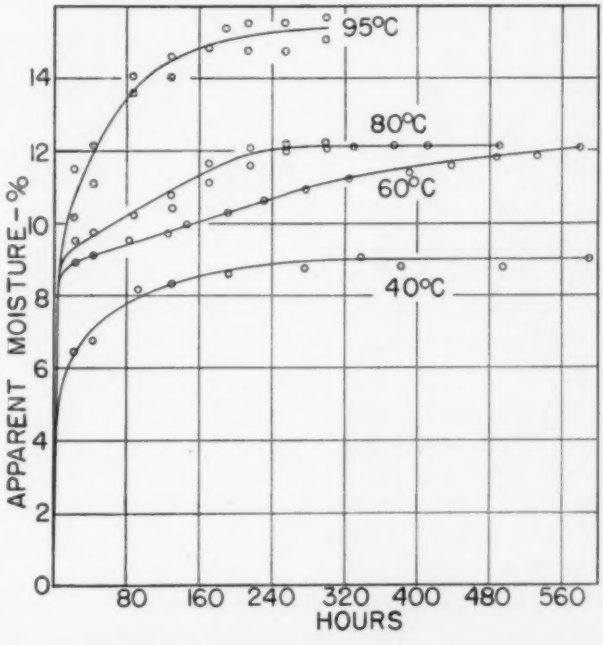

Fig. 1. Time-temperature drying curves for gluten feed—De Bruyn apparatus.

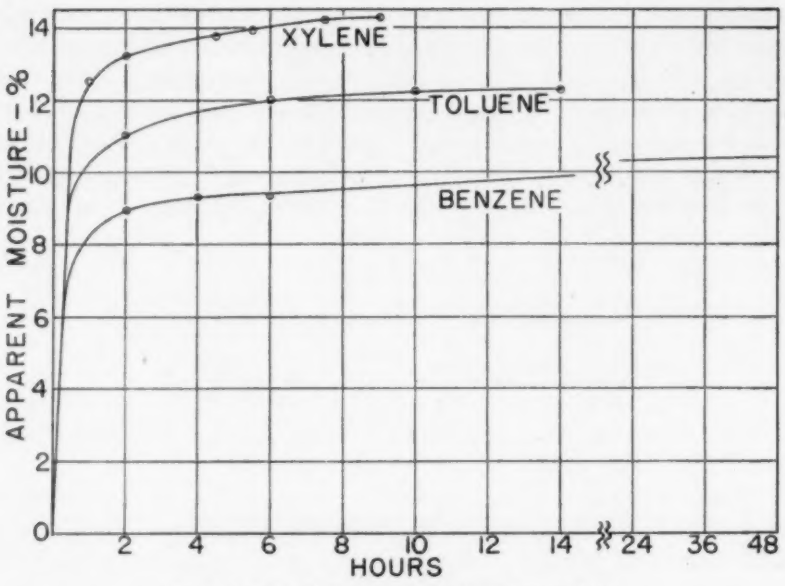

Fig. 2. Moisture of gluten feeds by distillation methods.

TABLE I

THE MOISTURE IN GLUTEN FEED

Moisture method	Temperature	Period of drying[1]	Apparent moisture	
			Range during the interval of drying	Final value
	°C	hrs	%	%
De Bruyn	40	280–600	8.80– 9.00	9.00
	60	280–600	11.00–12.05	12.05
	80	240–480	12.15–12.15	12.15
	95	160–280	14.80–15.34	15.34
Distillation	Benzene—bp 80°C	36– 48	10.33–10.33	10.33
	Toluene—bp 110°C	6– 14	12.00–12.25	12.25
	Xylene—bp 140°C	5– 7	14.00–14.30	14.30

[1] Weighings were made at regular intervals during the drying period.

in a specially designed apparatus previously described, which permits the drying of the material under high vacuum over P_2O_5. The flask containing the feed was placed in an insulated box containing the desired number of electric lights, while the flask containing the P_2O_5 was placed in a trough of running water. The temperatures were maintained within 3°C.

As shown in Table I, the apparent moisture of the feed varies from 9.00% to 15.34%. The high values obtained with the De Bruyn at 95°C (15.34%) undoubtedly included losses due to decomposition

as evidenced by the marked discoloration of the feed and the P_2O_5. Similarly, xylene distillation caused a marked charred appearance in the feed. It will be noted that the toluene distillation values agree reasonably well with the results obtained by drying the feeds in the De Bruyn at 60° and 80°C. Only a slight color formation occurred to the feeds dried in the De Bruyn and a somewhat more marked color occurred to the feed distilled with toluene. In view of this close agreement, it was first thought that possibly the true moisture content of the feed was in the region of 12.0%. The low value in the De Bruyn at 40°C (9.00%) was attributed to severe case-hardening. However, further work indicated that the agreement between the toluene distillation method and the De Bruyn at 60° and 80°C was quite fortuitous, or if not at least caused by the decomposition of some specific fraction of the feed. This was proven by the following experiments.

Use of Filter Aid in the Determination of Moisture in Gluten Feed

Work with steep water (paper II) indicated the necessity of dispersing the material in Hyflo Filter Aid prior to drying. This technique was applied to gluten feed, and by so doing the kiln-dried steep-water solids contained on the hulls were dispersed on the Filter Aid. Ten to 15 g of gluten feed was weighed into 50-ml beakers, to which approximately 20 ml of H_2O was added. The mixture was maintained at a temperature of 40°C for 2 hours, after which it was quantitatively transferred to previously dried De Bruyns containing Filter Aid. The De Bruyns were treated as described previously for steep water. The feeds so treated were then dried at 40° and 60°C along with control samples not subjected to the Filter Aid treatment. The results are shown in Figure 3 and tabulated in Table II, from which it can be observed that the Filter Aid treatment greatly facilitates the removal of the water.

When the softened moist feed was first dispersed on Filter Aid, it can be noted that at 40°C the apparent moisture value increased from 9.00% to 10.00%. The effect of case-hardening is very pronounced with gluten feed, and if not recognized a false equilibrium is obtained. The drying of gluten feed alone at 60°C resulted in a gradual weight loss over the total drying period until a value of 12.05% was reached. No indication of a break in the drying curve was obtained. However, if the feed is first softened by water and then dispersed on Filter Aid, a ready weight loss occurs during the first 90 hours of drying, after which complete constancy is reached for over a 100-hour drying period. Further heating causes an additional weight loss. It will be noted that at 60°C the feed mixed with Filter

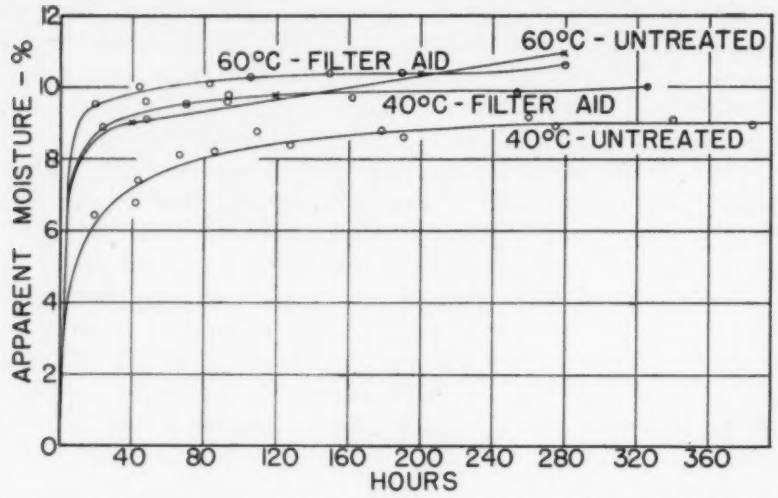

Fig. 3. The effect of Filter Aid dispersion on the determination of moisture in gluten feed.

TABLE II

USE OF FILTER AID IN THE DETERMINATION OF MOISTURE IN GLUTEN FEED

De Bruyns	Period of drying	Apparent moisture: Gluten feed alone—range during period of drying	Apparent moisture: Gluten feed plus Filter Aid—range during period of drying
	hrs	%	%
40°C	160–400	8.68– 9.00	9.85–10.00
60°C	90–200	9.50–10.40[1]	10.37–10.37

[1] Continued heating resulted in a continued gradual weight loss with no evidence of constancy until an apparent moisture value of 12.05% was reached.

Aid reached constancy at 10.37% within 90 hours, whereas it required 200 hours for the untreated feed to reach the same value. The results suggest that a slow decomposition occurs at this temperature, and that the prolongation in drying necessary for the untreated feed results in such a slow loss of moisture that it cannot be distinguished from the decomposition loss that later occurs. With Filter Aid, however, the moisture is lost so readily that a constancy period can be obtained before decomposition sets in. In the light of these data, it appeared that the moisture range of 10.3%–10.4% was of significance. It will be recalled that the value obtained at constancy by benzene distillation was 10.33%.

Oven Data with Gluten Feed

Further studies by the use of oven methods lent strong support in the determination of the true moisture content of the gluten feed. The feed was dried *in vacuo* at 80° and 100°C and also in the air oven

at 100°C. The data are shown in Figure 4. It is of interest to note that the air and vacuum oven methods at 100°C yielded essentially similar results. The feed showed a continued weight loss over the total 27-hour drying period and an apparent moisture value of 13.0%

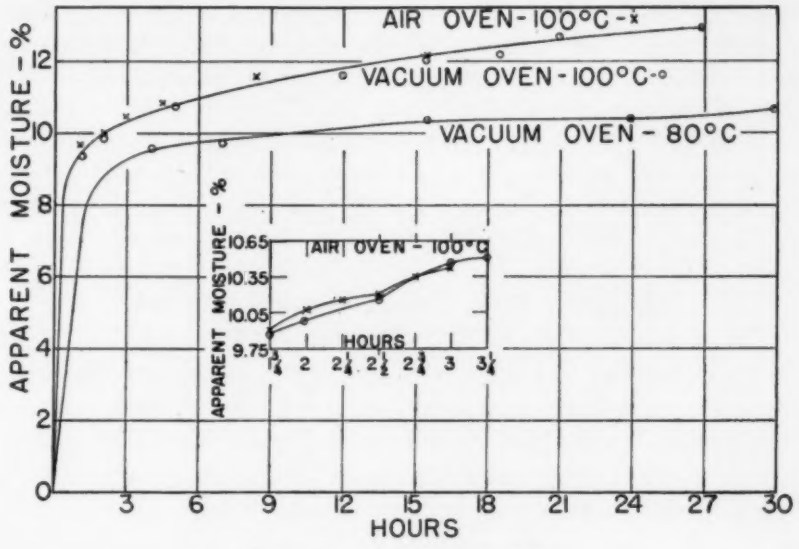

Fig. 4. Oven drying of gluten feed.

was reached. At 80°C *in vacuo* the results appeared to be of more significance. A period of essential constancy was obtained after 16 hours of drying, followed by a more marked weight loss. This is shown in Table III.

TABLE III

VACUUM DRYING OF GLUTEN FEED AT 80°C

Length of drying	Apparent moisture Duplicates	Apparent moisture Average	Weight loss per g of feed per hr
hrs.	%	%	*mg*
4	9.55– 9.53	9.54	23.7
7	9.78– 9.74	9.76	0.73
16	10.38–10.32	10.35	0.64
24	10.42–10.44	10.43	0.10
30	10.80–10.69	10.75	0.54

After 16 hours of drying, a distinct break occurred in the vacuum oven drying curve at 80°C and an additional period of 8 hours resulted in only a slight weight loss. The apparent moisture range during this period was 10.35%–10.43%, which is in agreement with the De Bruyn at 60°C where constancy was reached for 100 hours at 10.37%, and with the constant benzene distillation value of 10.33%.

More Detailed Study of the 100°C Air Oven Curve

The drying of the gluten feed at 100°C in both air and vacuum at hourly intervals indicated no breaks in the drying curve in the region of 10.40%. Later studies indicated that a break actually does occur in this region if samples are removed at 15-minute intervals through this critical moisture range. The results of two separate tests are shown in Figure 4 (inset) where it will be noted that a distinct break occurs when the apparent moisture reaches 10.2%. Reference to the curve will show that if these samples had been dried for half-hour intervals instead of 15-minute intervals, the break would probably have been overlooked. It is believed that this abrupt increase in loss after 2½ hours indicates the inception of decomposition.

Discussion of the Results Obtained with the First Sample of Gluten Feed

Three different moisture methods yielded essentially the same moisture value for the first sample of gluten feed studied. In two cases, complete constancy was reached for a considerable period, whereas in the third (vacuum oven 80°C) the constancy was not altogether complete and was of short duration.

Method	Apparent moisture %
Benzene distillation	10.33
Vacuum oven (80°C—20 hrs)	10.39
De Bruyn (60°C—Filter Aid)	10.37

It was also found that a break occurs in the drying curve at 100°C at the 10.20% level. By drying the feed in admixture with Filter Aid at 40°C, a value of 10.0% was obtained.

The above studies were conducted prior to the investigation carried out with steep water, hulls, and corn gluten, which are the components used in the preparation of gluten feed. These three components have been dealt with in a separate paper of this series (paper III), from which it was indicated that steep water is markedly unstable and that drying at higher temperatures or toluene distillation results in a marked decomposition. The benzene distillation method was found suitable for the determination of moisture in steep water and has been accepted as the official reference method by the T. A. C. of the C. I. R. F. The results for gluten feed given above indicated that the benzene distillation method should prove equally suitable for gluten feed. Studies with other feed samples indicated this belief to be valid. The results obtained are given in the following sections.

Further Moisture Studies with Gluten Feed

In view of the agreement obtained by the use of the benzene distillation method, the Filter Aid De Bruyn method at 60°C, and the vacuum oven method at 80°C for the first sample of gluten feed studied, the work was repeated with three additional commercial samples ground to pass a 20-mesh sieve. The results are shown in Table IV. An immiscible solvent boiling at a temperature lower than benzene was also included, as well as one of the moisture methods used in the corn industry.

TABLE IV

THE MOISTURE CONTENT OF GLUTEN FEED

Moisture method	Period of drying	Sample I	Sample II	Sample III
	hrs	%	%	%
Benzene[1] distillation, 30-g samples	17	8.13[2]	7.73	8.33
	20	8.20	8.07	8.97
	25	8.27	8.07	9.00
	41	8.33	8.07	9.07
	48	8.33	—	9.07
	60	—	—	9.07
Vacuum oven, 80°C	16	8.52	7.99	9.08
	24	8.71	8.16	9.30
Average	—	8.62	8.08	9.19
De Bruyn, 60°C, dispersed on Filter Aid	60	8.05	7.82	9.01
	82	8.50	7.94	9.09
	104	8.57	8.04	9.13
	126	8.66	8.10	9.19
	148	8.74	8.15	9.25
	170	8.87	8.17	9.29
Average		8.67	8.08	9.19
Skelly-Solve (petroleum ether bp 55°–70°C)	70	6.96	6.66	8.27
Union Starch method	—	9.38	8.66	9.74

[1] A correction of 0.02 ml is required when using benzene.
[2] Intermediate values were taken during the course of the distillation and are only approximate.

The three feeds showed some variation from the first composite sample used. In no case was absolute constancy reached with the De Bruyns at 60°C, the feeds exhibiting a continued weight loss of 0.03% to 0.10% daily after the first 82-hour drying periods. As a result, an approximation is necessary in determining the moisture content by the De Bruyn method. This was done by averaging the values between the 82- and 170-hour drying period. These results

are then in agreement for samples No. 2 and No. 3, with the direct moisture values obtained by benzene distillation and also with the average of the 16- and 24-hour values *in vacuo* at 80°C. The distillation results for sample No. 1 were low as compared to both the De Bruyn and vacuum oven at 80°C. Later work has suggested that this may have been caused by very severe case-hardening. It will be noted that Skelly-Solve, which boils at 55°–70°C, is quite inadequate for the removal of the total water—underestimating the moisture by 0.9%–1.7%. The commercial method, as compared to the benzene distillation values, overestimates the moisture by 0.6%–1.0%.

In view of the De Bruyn results at 60°C, which yielded no constant value for any period, thus suggesting a slow decomposition, the work was repeated with four additional samples, but in this case the De Bruyn tests were conducted at 50°C rather than 60°C. By so doing it will be noted from Table V that a definite plateau is obtained which is in agreement with the benzene distillation results.

TABLE V

THE MOISTURE CONTENT OF GLUTEN FEED

Sample No.	Benzene distillation (constancy—42 hrs)	De Bruyn—50°C		
		Drying period of constancy[1]	Moisture range	Final moisture value (average)
	%	*hrs*	%	%
1	7.60	370–505	7.53–7.55	7.55
2	9.80	370–525	9.71–9.82	9.78
3	9.90	370–620	9.80–9.92	9.87
4	9.10	460–620	9.12–9.17	9.14

[1] Weighings made every 48 hours. The vacuums were maintained at less than 0.1 mm.

The results in Table V added further confirmation to the previous data. In one case, water is determined directly, and in the second, the feed is dried to constancy in high vacuum over P_2O_5 after dispersion on Filter Aid, at the maximum temperature allowable. The results agree, as shown above, within 0.1% for the four feeds tested. These data, in conjunction with the results previously reported for steep water and hulls (paper III), indicated that either the benzene distillation method or the De Bruyn procedure of softening the feed and dispersing it on Filter Aid before drying to constancy over P_2O_5 at 50°C would prove equally suitable as reference methods for the determination of moisture in gluten feed. Further work indicated, however, that the De Bruyn procedure would be quite unsuitable since in the feeds produced by some of the members of the industry, a considerable quantity of volatiles are present, which volatilize in the De Bruyn and, as a consequence, yield moisture values which

are too high. This was found to be true also for hydrol and molasses feeds. Typical data obtained for the feeds produced by several members of the industry are given in Table VI.

TABLE VI

THE MOISTURE OF MOLASSES, HYDROL, AND GLUTEN FEEDS

Product	Benzene distillation	Moisture method De Bruyns at 50°C, Filter Aid 400–700 hrs	Air oven, 100°C 3 hrs
Gluten feed—No. 1	7.60	8.10	7.69
Gluten feed—No. 2	7.20	7.65	7.26
Gluten feed—No. 3	9.94	10.84	10.05
Gluten feed—No. 4	10.58	11.25[1]	10.40
Gluten feed—No. 5	11.18	11.25	11.05
Hydrol feed	8.03	8.13	8.12
Sweetened feed (15%)	9.76	9.91	9.70
Sweetened feed (20%)	8.76	9.40	9.20

[1] Volatiles equivalent to 0.25% were isolated from this sample by the use of dry ice.

It will be noted that the three-hour air-oven method at 100°C, which was previously found to be in fair agreement with the benzene distillation, is also in agreement for the gluten feed samples given in Table VI. The De Bruyn procedure of drying at 50°C, however, yields results which are 0.10% to 0.90% higher than the benzene distillation values. That this discrepancy is not largely due to severe case-hardening which prevents the liberation of moisture by benzene distillation was evident by the presence of volatiles which were noted in the connecting tube between the feed sample and the P_2O_5 flask. In one case, by a relatively crude method, 0.25% of volatiles was isolated. This variability in feed is to be expected in view of the method of preparation. Some companies immediately concentrate the steep water, leaving the steeping tanks to the desired Baumé, while others allow the steep water to go through an initial fermentation period. By so doing, they have found that a higher concentration of solids can be obtained in the steep water without tube incrustation. In other cases some of the effluents from the modified starch products are added to the steep water. As a result the variability found with respect to volatiles in the different feeds is to be expected. In view of this variability, the only method considered suitable as a reference procedure for the determination of the true moisture in gluten feed was the benzene distillation procedure. It is recognized that this method may, in isolated cases, yield low values because of unduly severe case-hardening. Further work has shown that in certain cases constancy during distillation is not attained for 70 to 100 hours. However, the results so far obtained have indicated that this error will be of reasonably small magnitude. In any event the work shows

that no indirect moisture method could prove suitable for products of the nature of gluten, hydrol, and sweetened feeds.

Relation of Storage to Moisture in Gluten Feed

During these studies samples were stored for relatively long periods. In certain cases, to conserve space in the refrigerator, the samples were allowed to stand at room temperature which, during the summer months, ranged from 90° to 100°F. With storage a noticeable darkening in the color of the feed occurred. Studies were conducted to determine whether this color change was accompanied by an actual DS loss in the feed, and whether as a result, the apparent moisture of feed would increase with storage.

Studies have been conducted covering the effect of moisture content, of pH, and of temperature of storage on the keeping qualities of gluten feed. Only that work pertinent to the moisture problem of gluten feed will be dealt with here. The results of a single typical study are given.

Samples of a thoroughly mixed sample of gluten feed (100 g) were placed in one-pound friction-top tins and stored for varying lengths of time at different temperatures. Considerable pressure developed in the tins during storage at the higher temperatures and as a result some leakage occurred (0.2%–0.4%). This loss was prevented in subsequent work by soldering the lids. Upon completion of the storage period, the samples were transferred to the refrigerator until required.

A moisture analysis of the products subjected to the various storage treatments was conducted by the following procedures:

De Bruyn, Filter Aid—50°C: The dry substance present in the sample used for the storage study was determined by drying the feed to constancy, after admixture with Filter Aid, over P_2O_5 at 50°C in De Bruyns. The feed contained 90.54% dry substance (9.46% moisture). The samples stored for the different periods were then dried under similar conditions, and moisture values greater than 9.46% can be attributed to changes caused by the storage treatment. This method gives a measure of the dry substance loss. This study was the first application of the reversibility method (paper IV) to the determination of moisture in feeds or cereals.

Benzene distillation: The moisture content of the feed, as determined by the benzene distillation method, was 9.45%. This value is in agreement with the De Bruyn results, indicating that this particular feed contained no volatiles that will volatilize under the conditions used in the De Bruyns. The moisture contents of the various stored feeds were determined by the benzene distillation procedure. Values

in excess of 9.45% can be attributed to the formation of water of decomposition. A comparison of the distillation and De Bruyn results shows the relation between water of decomposition and the total dry substance loss.

The results of the storage study are given in Table VII. These results indicate the need of storing gluten feed at low temperatures.

TABLE VII

STORAGE AND MOISTURE OF GLUTEN FEED

Condition of storage	Apparent moisture			
	Benzene distillation—72 hrs		De Bruyn—50°C,[1] 500 hrs	
	Moisture	Water of decomposition	Moisture	Dry substance loss
	%	%	%	%
Control	9.45	—	9.46	—
4 wks at 38°C	9.68	0.23	9.89	0.43
2 wks at 50°C	10.41	0.96	10.42	0.96
4 wks at 50°C	10.60	1.15	10.70	1.24
2 wks at 60°C	10.84	1.39	11.14	1.68

[1] Constancy in weight during the last 150 hours of drying.

Four weeks of storage at 38°C (100°F) resulted in a dry substance loss of 0.43%, of which 53.5% was due to water of decomposition. Two weeks at 50°C (120°F) caused a dry-substance loss of 0.96%, which could be wholly attributed to decomposition due to the splitting off of water. This decomposition was accompanied by an increasing discoloration of the feed. The results demonstrate the thermolability of gluten feed and show that the major product of decomposition is water. Later work has indicated that 10% to 15% of the total dry-substance loss is attributable to the formation of gas, the nature of which has not been determined.

As pointed out in a previous paper (II) steep water, which is one of the major constituents of gluten feed, is largely composed of nitrogenous hydrolytic products, ash, and reducing sugars. Some ammonia nitrogen is also present. During storage some of these products apparently condense with the splitting off of water. If the feed is stored with a low moisture content (3%), the decomposition is greatly lessened, indicating that in this case insufficient water is present to allow for the movement of the interacting constituents.

The results obtained in the storage study add further confirmation to the use of the benzene distillation method for the determination of moisture in a product such as gluten feed. It is of interest to note the parallelism that occurs between determining the moisture directly and the results obtained by the indirect procedure (De Bruyn).

The data obtained by the use of the benzene distillation method and the De Bruyn method in the study of decomposition of gluten feed suggests that these procedures might have a wide application. They may prove of value in the approach to studies dealing with the interaction of sugars and nitrogenous products, or for that matter to any condensation reaction, resulting in the splitting off of water. The relation of water formation to the total dry substance changes can be determined with precision.

Summary

The Technical Advisory Committee of the C. I. R. F. has accepted the benzene distillation method, carried to completion, as the official reference moisture method for the determination of moisture in corn gluten feed, sweetened and unsweetened.

The results of these investigations suggest that the benzene distillation method should have a wide utility for the determination of moisture in thermolabile products, such as hydrolytic protein mixtures. It should also prove suitable for cereal products such as malt, distiller's and brewer's grain, and other processed products which may be unstable to heat.

THE DETERMINATION OF MOISTURE IN THE WET MILLING INDUSTRY. VI. CORN OIL MEAL

L. Sair[1] and W. R. Fetzer[2]

This paper on corn oil meal completes the series dealing with the moisture in corn and its feed by-products. The germ floating off in the wet milling process is thoroughly washed, passed through a moisture expeller, and is then dried in kilns to a moisture value approximating 1.0%. It is then preheated to approximately 95°C before passing through an oil expeller which exerts a pressure of approximately 4,000 pounds per square inch in removing the oil. The ground expeller cake is sold under the trade name of corn oil meal.

A moisture study was made on a single composite sample of corn oil meal. The procedures used included distillation methods, De Bruyn method, oven methods, and reversibility studies. The procedure of sampling and the experimental details were similar to those described for corn (No. I of this series). The meal as received from the plant is ground fine and completely passes through a 20-mesh sieve. Seventy percent of the material passes through a 30-mesh

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sieve. Routine analysis of the corn oil meal used in this study yielded the following results:

	%
Moisture	10.1
Protein (N × 6.25)	23.5
Ether extract	10.0
Crude fiber	9.2
Ash	2.5
NFE (by difference)	44.7
Acidity as HCl	0.52
pH	4.6

Moisture Results

The moisture results obtained by the use of distillation methods, De Bruyn methods, and by oven methods are given in Table I, which again illustrates the variation in moisture values with different methods. The moisture range varied from 9.94% to 10.69%. The results in Table I show that the obtained moisture values fall roughly into three groupings. The lower moisture range of 9.94% to 10.17% was obtained by drying the meal in an air oven at 100°C for 72 hours (9.94%), by drying in a De Bruyn over P_2O_5 at high vacuum at 60°C for 272 hours (10.17%), by drying in the vacuum oven at 80°C for 120 hours (10.04%), and by benzene distillation (10.12%). The highest value was obtained by vacuum drying at 115°C for 40 hours (10.69%). The intermediate values were obtained by toluene distillation (10.42%), by De Bruyn drying at 90°C (10.36%), and by vacuum drying at 100°C for 40 hours (10.36%) and for 120 hours (10.46%).

TABLE I

MOISTURE OF CORN OIL MEAL BY VARIOUS METHODS

Moisture method	Temperature	Length of drying, showing period of essential constancy	Moisture range during period of essential constancy	Final moisture value	
				Duplicates	Average
	°C	*hrs*	%	%	%
De Bruyn	60	132–272	10.16–10.17	10.18–10.16	10.17
	90	88–272	10.33–10.37	10.36	10.36
Oven drying	*Vacuo* 80	40–120	10.02–10.04	10.04–10.05	10.04
	(1) 100	18– 40	10.24–10.36[1]	10.36–10.35	10.36
	(2) 100	40–120	10.28–10.46[1]	10.46–10.46	10.46
	115	8– 40	10.41–10.69[1]	10.68–10.70	10.69
	Air 100	18– 72	9.90– 9.94	9.97– 9.92	9.94
Distillation	Benzene	21– 68	10.14–10.12	10.08–10.16	10.12
	Toluene	21– 45	10.42	10.49–10.29–10.43 10.47	10.42

[1] No period of constancy reached in the vacuum oven at 100° and 115°C.

The results indicate a different moisture value for almost every method used. Vacuum oven drying at 80°, 100°, and 115°C yielded respectively 10.04%, 10.41%, and 10.69%. The De Bruyns at 60° and 90°C yielded a difference of 0.19%. The results indicate an increasing moisture value with increase in temperature or severity of drying conditions, and from the obtained data it is impossible to make any deductions regarding the true moisture content of the corn oil meal. It would be difficult to state with any degree of confidence the true moisture content of the feed. The samples dried under the various oven conditions were used for reversibility studies, and the obtained results have added further confirmation to the utility of this procedure in the clarification of the moisture problem. A full discussion of the reversibility method has been previously given (IV, this series).

Reversibility Studies with Corn Oil Meal

The samples dried in the vacuum oven for varying intervals were placed in the air oven at 100°C for 24 hours. The meal adsorbed water under these conditions in accordance with its extent of dryness. The data are shown in Table II.

TABLE II

REVERSIBILITY AND MOISTURE IN CORN OIL MEAL

Drying treatment	Apparent moisture	Reversible experiment: Reversible moisture values—air oven—100°C	Adsorption
	%	%	%
Vacuo 5 hrs	10.12	9.98 10.02 Av 10.00	0.12
18 hrs	10.24	10.02 10.01 Av 10.02	0.22
24 hrs	10.28	10.04 10.00 Av 10.01	0.26
40 hrs	10.36	10.02 9.99 Av 10.00	0.36

The results indicate that continued drying of corn oil meal for 40 hours results in a continued loss of water, as indicated by the fact that the samples yielded the same reversible moisture value. A continued increase in adsorption occurred with drying period, showing that no constancy was yet attained with respect to moisture removal. The results of this experiment suggested that the moisture content of corn oil meal was in excess of 10.36%.

There are several surprising points of interest in these results: Although the meal was heated for 40 hours *in vacuo* at 100°C, apparently no loss in volatiles occurred, notwithstanding the fact that the feed contained 10.0% of crude fat. Similar drying treatment with corn (paper II) and corn gluten (paper III) resulted in a loss of volatiles equivalent to 0.59% and 1.91% (110°C vacuum, 45 hours) respectively, even though these products contained a much smaller percentage of ether extract. The results suggest that in the processing of corn oil meal the fat becomes firmly attached or is entrapped within the feed. That this probably occurs is evidenced by the tenacity with which corn oil meal retains its water. The granulation of the meal used was such that 70% passed through a 30-mesh sieve. Corn of 40-mesh loses its total moisture *in vacuo* at 100°C in a maximum of 16 hours. Gluten meal (20-mesh) attained constancy in weight within 16 hours. Yet corn oil meal continues to lose moisture after 40 hours of drying. The germs are dried in kilns to a low moisture content (1.0% or lower) and a pressure of approximately 4,000 pounds per square inch is used for the expression of the oil from the heated germs. Under these conditions, it appears as though a portion of both the fat and the moisture become firmly fixed in the feed. Additional evidence indicating that this occurs is suggested by the discrepancy between the benzene and toluene distillation results which differed by 0.30%. The maximum difference previously found for these solvents in the studies with corn, corn gluten, and corn gluten meal was 0.1%.

A second reversibility study was conducted with the samples dried *in vacuo* at 80° and 100°C. Under these drying conditions, the moisture value had reached two plateaus which differed by 0.4%. By placing the dishes containing the feed dried in the vacuum oven at 80°C into the vacuum oven at 100°C, an additional moisture loss occurred equivalent to that obtained by drying the feed directly in the vacuum oven at 100°C (Table III).

TABLE III

SECOND REVERSIBILITY STUDY WITH CORN OIL MEAL

Drying treatment	Apparent moisture	80°C vacuum samples placed *in vacuo* at 100°C		
		24 hrs	48 hrs	72 hrs
	%	%	%	%
Vacuo 80°C 84 hrs	10.02	10.34	10.38	10.43
Vacuo 100°C 96 hrs	10.42	—	—	—

The results in Table III suggested that a false equilibrium was obtained *in vacuo* at 80°C, and that constancy is no indication of the true moisture content. It seemed that severe case-hardening of corn

oil meal prevented the liberation of the total moisture. That this is the case is proved by the reversibility study reported in Table IV.

Table IV shows that vacuum drying at 100°C for 96 hours causes no appreciable decomposition in the corn oil meal, as evidenced by

TABLE IV

FURTHER STUDY OF REVERSIBILITY AND MOISTURE OF CORN OIL MEAL

Drying treatment	Apparent moisture	Reversible experiment	
		Reversible moisture value[1]	Adsorption
	%		%
Vacuo 80°C 68 hrs	9.97	10.14 10.09 Av 10.12	−0.15
Vacuo 80°C 44 hrs 100°C 72 hrs	10.43	10.15 10.16 Av 10.16	0.27
Vacuo 100°C 96 hrs	10.42	10.17 10.12 Av 10.14	0.28

[1] Samples moistened and re-dried for 48 hrs *in vacuo* at 60°C.

the fact that the feed reaches essentially the same reversible moisture value as does the feed dried *in vacuo* at 80°C. It is of interest to note that by moistening the feed dried *in vacuo* at 80°C, prior to redrying at 60°C *in vacuo*, the material lost rather than gained moisture. This result adds further confirmation to the evidence indicating that the low values obtained by vacuum drying at 80°C are attributable to case hardening.

The above results, taken in conjunction with those previously given, demonstrate that values in the lower moisture range—those obtained by benzene distillation, by the air oven at 100°C, by the vacuum oven at 80°C, and by the De Bruyn at 60°C—are lower than the amount of moisture actually present.

A third reversibility experiment was conducted with the samples dried *in vacuo* at 115°C, from which it is evident (Table V) that prolonged drying under those conditions results in the decomposition of the feed.

The meal dried for 2.5 hrs *in vacuo* at 115°C was used as the control sample. This was necessary in view of the severe case-hardening of corn oil meal, which results in moisture values for controls that are lower than would otherwise be obtained. This feature was previously dealt with with 10-mesh corn (II of this series). The results show that the vacuum drying of corn oil meal for 8 hours causes no loss in volatiles or decomposition, as evidenced by the fact that the meal sample is perfectly reversible to the value obtained by

TABLE V

THIRD STUDY OF REVERSIBILITY AND MOISTURE OF CORN OIL MEAL

Drying treatment, *vacuo*—115°C	Reversible experiment				
	A Apparent moisture	B Reversible moisture value[1]	C Adsorption	D[2] Decomposition B—10.00	E Calculated true moisture A–D
	%	%		%	%
2.5	10.31	9.98 10.02 Av 10.00	0.31	—	—
8	10.41	10.00 10.01 Av 10.00	0.41	—	10.41
16	10.50	10.08 10.12 Av 10.10	0.40	0.10	10.40
24	10.58	10.20 10.22 Av 10.21	0.37	0.21	10.37
40	10.69	10.36 10.34 Av 10.35	0.34	0.35	10.34 Av 11.38

[1] Samples redried for 48 hrs in the air oven at 100°C.
[2] Decomposition includes the loss of volatiles.

drying the feed for only 2–5 hours. The results show that an apparent moisture value of 10.41% does not include losses other than water. Beyond this value, however, decomposition does occur as evidenced by the disparity in the reversible values. It will be noted that the average calculated true moisture for this sample of corn oil meal is 10.38%.

The calculated moisture value by the reversibility study (10.38%) is in agreement with the De Bruyn method of drying to constancy at 90°C (10.36%) and with the toluene distillation method (10.42%). Studies with corn (II of this series), with corn gluten (III), and with corn gluten meal (IV) have shown that the toluene distillation procedure yields results in agreement with the reversibility method. The toluene distillation method has been accepted by the Technical Advisory Committee of the C. I. R. F. as the official reference method for the determination of moisture in these products, and the results of this study indicate that the toluene distillation method is also satisfactory for corn oil meal.

Reference to Figure 1 shows that it requires 80 to 90 hours *in vacuo* at 100°C to remove the total moisture from corn oil meal. This is in contrast to other products, which may lose their total moisture in 5 to 20 hours. This result again demonstrates the danger involved in attempting to generalize with a specific indirect procedure.

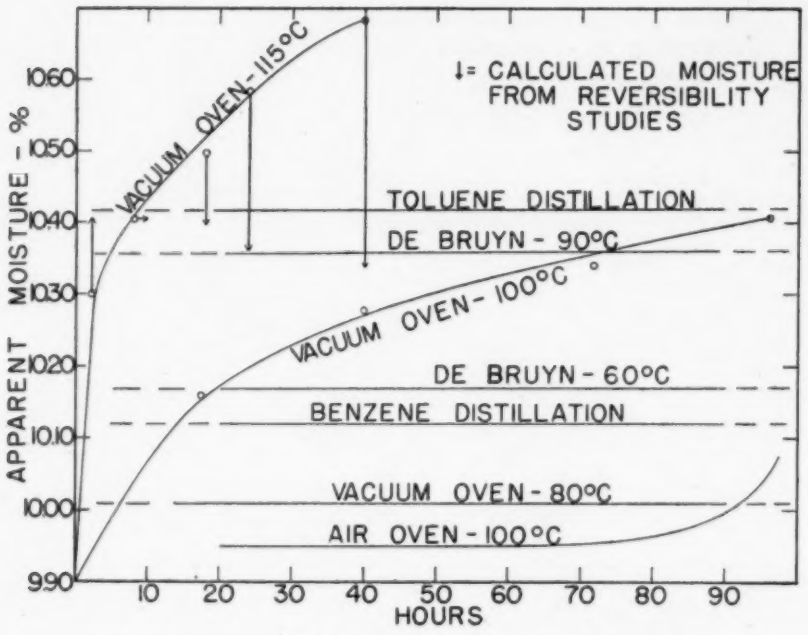

Fig. 1. The determination of moisture in corn oil meal by various methods.

Conclusion

The results of these investigations with corn and its feed by-products have led to the recommendation of two official reference moisture methods within the wet milling industry. Both methods determine moisture directly. One method (toluene distillation) is to be used with the stable products, which include corn, hulls, corn gluten, gluten meal, and corn oil meal. The second (benzene distillation) is used for the unstable products, which include gluten feed, steep water, and sweetened feeds prepared by the addition of either hydrol or molasses. These two procedures must be carried to completion, since otherwise values are obtained which must be termed empirical. The feeds must be ground to pass at least a 20-mesh sieve. With steep water it is necessary first to disperse the material on Hyflo Filter Aid. If the necessary recognition is given to the various factors which enter into a moisture determination, these two recommended procedures not only give true moisture values for the products produced in the wet milling industry, but we believe they could be extended to similar moisture determinations in other industries.

The usual indirect moisture methods can only yield relative results. Cereals and feeds differ in the amount of volatiles present, in the tenacity with which water is held, and even in stability. For rapid

moisture tests the oven procedures undoubtedly are of value, but they cannot be considered as absolute methods, and as such should not be termed official methods. The official desiccator method, which makes use of sulfuric acid as a desiccant, does not recognize the very important factor of case-hardening in feeds and cereals. Moreover, it is difficult to maintain a suitable vacuum in the desiccators. The results of these investigations would indicate that this procedure will only yield relative results.

The results of these investigations indicate that the two distillation procedures, the benzene and toluene distillation methods carried to completion, are the only present satisfactory distillation methods for the determination of the true moisture in cereals and feeds.

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APPENDIX

OFFICIAL REFERENCE METHOD OF THE CORN INDUSTRIES RESEARCH FOUNDATION FOR THE DETERMINATION OF MOISTURE IN CORN

Toluene Distillation Method

APPARATUS

Mill: Wiley Laboratory Mill: Intermediate Model No. 4267 P, with necessary accessories. Fisher Scientific Co. (1 extra 20-mesh sieve). The mill as received should be changed as follows: Drill a 1/4″ hole in the lid of the hopper. Prepare a plunger of hard wood 6″ long, 2″ of the length being 1/2″ in diameter and the remainder a little less than 1/4″.

Weighing bottles: 60 × 40 mm Pyrex, standard taper 40/12; stopper also used with distillation flasks described below.

Distillation apparatus: Blueprints covering the apparatus used are on file with the S.G.A. Company or a blueprint may be obtained from the Corn Industries Research Foundation, 5 East 45th St., New York City. The individual pieces are as follows:

Erlenmeyer flasks: 250-ml Pyrex standard taper 40/50.

Distillation traps: Pyrex standard taper 40/50—24/40, 5-ml capacity graduated in 1/10 ml. The connecting tube between the 40/50 joint and the trap proper should be insulated. A satisfactory method is to wrap this tube with at least three layers of electricians' tape, overlapping each turn, followed by two coats of heavy shellac.

Condensers: Pyrex, straight tube, similar to West type, 40-cm jacket, water tubes on same side, 24/40 standard taper water-cooled joint, lower end; fitted with drip tip with ring seal, the tip being opposite to water tube. The top of the condenser is an extension of the inner tube, 10 cm long.

Stainless steel baths: Dia 4″, depth 4″, home-made.

REAGENTS

Asbestos: Medium fiber, acid washed.

Toluene: Reagent grade.

PREPARATION OF APPARATUS

1. *Distillation flasks and asbestos:* Distillation flasks containing 5 to 8 g of asbestos are dried overnight in an air oven at 100°C. The flasks, which are first stoppered, are cooled in a desiccator containing calcium carbide or calcium chloride. To prevent the freezing of the glass stopper a thin strip of paper is inserted between it and the flask.

2. *Cleaning of traps and condensers:* The traps and condensers must be scrupulously clean. The traps are first thoroughly scrubbed with water and cleanser. A small narrow brush is necessary to reach to the bottom of the trap. At the end of this stage no droplets of water should stick to the inner sides of the traps. Hot fresh cleaning solution is then added and the traps are left for periods ranging from two hours to overnight. Glass stoppers standard taper 40/50 are much more satisfactory than rubber stoppers for closure of the standard taper 40/50 end of the trap. The traps are then thoroughly rinsed with distilled water and are refilled with a 1% solution of caustic soda. After 15 to 30 minutes the traps are again very thoroughly rinsed. Drying of the traps has been carried out by inverting them on a towel, and leaving them upright overnight. A second towel is placed above to prevent dust accumulation. If desired the drying operation can be hastened by the use of an oven. The condensers are cleaned in the same manner except that the water-cleanser treatment is not used.

PREPARATION OF SAMPLE

1. *The sampling of corn:* Every care must be taken to insure uniformity in the sample of corn under test. Whole corn kernels of uneven moisture require considerable time to reach equilibrium, much more so than when the material is in the ground state. It is necessary, therefore, if only a small sample is available, to shake the corn for some time in a bottle filled to one-third capacity. If a large sample is used, then some form of commercial sampler such as the Boerner sampler should be used.

2. *The moisture of corn:* If the moisture content of the corn is too high to be directly ground in the Wiley, it is necessary first to pre-dry the material.

Fill previously tared moisture dishes (with covers) with the damp grain and weigh. Place the dishes (cover removed) in a warm, well ventilated place (preferably on top of heated oven) protected from dust so that the grain will dry reasonably fast and reach an approximate air-dry condition in from 14 to 24 hours. Weigh the dishes and calculate the percentage of moisture lost in air-drying. Calculate the percentage of total moisture in original sample as follows:

Total moisture ..... = $A + (100 - A)B/100$,
where total moisture ..... = % total moisture
A ..... = % moisture lost in air-drying
B ..... = % moisture in air-drying sample as determined by toluene distillation.

With corn of usual moisture content (10% to 20% or possibly higher) this pre-drying operation is unnecessary; 20 to 30 g of corn,

depending on its moisture content, is weighed in a glass-stoppered weighing bottle.

MILL PROCEDURE

The weighed corn is ground quantitatively in a small Wiley mill. The Wiley mill is of the shearing type and during grinding very little heating occurs. The procedure of grinding is as follows: The corn is transferred to the closed hopper, and the lid is replaced. The 20-mesh sieve, connected to a glass receiving bottle, is fixed in position. The small glass plate is tightly screwed to the face of the mill. A clean sheet of paper is placed under the receiving bottle in order that any falling particles may be recovered.

After the motor has been turned on, the wooden plug which seats the hopper is raised. The raising and lowering of this plug facilitates the steady flow of the kernels. No kernels should be allowed to enter the mill unless the motor is on, since otherwise the mill will become plugged.

Upon completion of the grinding, the mill is carefully disassembled. Any dust in the hopper is brushed into the mill with a camel's hair brush. The dust accumulating on the rim of the glass plate is brushed on to the sheet of paper. The mill is then brushed completely, care being taken to insure that all particles between the knife plates are removed. The ground material is retransferred to the weighing bottle which contained the whole corn. The moisture loss during grinding is determined. This value will vary with the moisture of the corn, the outside temperature, etc. Typical results obtained are as follows:

Sample	Moisture of corn	Grinding loss, %, duplicates
1	13.66	0.38; 0.37
2	14.18	0.30; 0.29
3	16.14	0.44; 0.45
4	17.58	0.57; 0.60

DETERMINATION OF MOISTURE

The ground corn is transferred to the distillation flask and is washed out with increments of toluene to insure a complete transfer. Approximately 75 ml of toluene is added. The apparatus is then connected.

TREATMENT OF JOINTS OF THE ASSEMBLY

A few drops of xylene are used to seal the glass joints between the flask, trap, and condenser. A few stiff turns seal the joints tightly. As a precautionary measure it has been found advisable also to seal the top connection between the trap and condenser with a few turns of electrician's tape. The traps are filled with toluene before assembly.

With these precautions, distillation can be continued for days without any appreciable leakage.

CONDENSERS

A steady flow of water is at all times maintained during the distillation. An inverted glass test tube, which fits snugly on the top of the condenser, is used to reduce to a minimum the movement of air to and from the condenser.

OIL BATH

The oil baths are small stainless steel (or copper) containers with a screen insert one inch from the bottom, filled with corn oil. To prevent undue heat loss during distillation the baths have been insulated. An outer metal shield covered with asbestos fits snugly (within $\frac{1}{8}''$) around the oil baths. The baths are also covered during distillation with cardboard containing an opening for the neck of the flasks.

DISTILLATION

After the apparatus is completely assembled the heaters are turned on. Distillation is started quite slowly (1 to 2 drops per second) but can be hastened after the first half-hour period to about 2 to 4 drops per second. The distillation is so conducted that the distillate is condensed in the bottom two inches of the condenser. After the first hour, and then from time to time, a few milliliters of toluene is poured into the top of the condenser to wash down any droplets that may have formed inside the tube. If the apparatus is properly cleaned it will be found that a clean, round meniscus is formed and that no water or only a trace sticks to the sides of the trap. Distillation should be continued for 48 hours.

TREATMENT OF THE TRAPS

Before the apparatus is disassembled, the condenser should be thoroughly washed down with toluene. If properly cleaned, no droplets of water should stick to the inside tube, and the condenser tube surface, if examined under the light, should be completely free of contamination.

A small piece of clean rubber tubing is attached to the end of a stiff wire and this is used for thoroughly scrubbing down the sides of the traps so that any occluded water may settle. The traps are then placed in water at 68°F to reach equilibrium (1 to 2 hrs) before reading.

CORRECTION FACTOR

A value of 0.03 ml is added to the obtained water value to compensate for the water that remains in the toluene.

FURTHER DISTILLATION

Since in some cases it has been found that 48 hours of distillation is insufficient it is advisable to reassemble the apparatus and continue the distillation for another period. Experience may indicate that this is unnecessary under most circumstances. The course of the distillation can be followed closely by taking readings during the distillation.

CALCULATIONS

Sample wt of corn	25.4995 g
Wt ground corn	25.3861 g
Moisture loss during grinding	0.1134 g
Ml of water obtained	3.99
Ml of water plus correction (0.03)	4.02
Total moisture (4.02 + 0.1134)	4.1334 g
Moisture, %	16.21

OFFICIAL REFERENCE METHOD OF THE CORN INDUSTRIES RESEARCH FOUNDATION FOR THE DETERMINATION OF MOISTURE IN CORN GLUTEN MEAL AND CORN OIL MEAL

Toluene Distillation Method

APPARATUS AND REAGENTS

Similar to those described for corn.

PREPARATION OF APPARATUS

Similar to that described for corn.

PREPARATION OF SAMPLE

Similar to that described for corn.

MILL PROCEDURE

Commercial corn oil meal is usually ground to pass 20 mesh, and further laboratory treatment is unnecessary. Gluten meal must be quantitatively ground, as described for corn, since a portion of the gluten meal dries into hard, horny pellets of varying sizes.

DETERMINATION OF MOISTURE

The procedure used is similar to that described for corn.

OFFICIAL REFERENCE METHOD OF THE CORN INDUSTRIES RESEARCH FOUNDATION FOR THE DETERMINATION OF MOISTURE IN CORN GLUTEN FEED AND SWEETENED FEEDS

Benzene Distillation Method

APPARATUS

Similar to that described for corn.

REAGENT

Benzene, reagent grade.

PREPARATION OF APPARATUS

Similar to that described for corn.

PREPARATION OF SAMPLE

Similar to that described for corn.

MILL PROCEDURE

The gluten feed should be ground quantitatively as described for corn.

DETERMINATION OF MOISTURE

The procedure used is similar to that described for corn. A correction factor of 0.02 ml is added to the obtained water value rather than 0.03 ml, which is used for toluene. Forty-eight hours of distillation is the minimum time required for the complete removal of water, and in certain cases distillation must be prolonged for periods ranging up to 80 hours.

OFFICIAL REFERENCE METHOD FOR THE DETERMINATION OF MOISTURE IN STEEP WATER

Benzene Distillation Method

APPARATUS

Distillation apparatus: Similar to that described for corn.

Test tubes: Pyrex 100 × 13 mm. Used as pestles for incorporating the steep water into the Filter Aid.

REAGENTS

Benzene: Reagent grade.

Filter Aid: Hyflo, Johns Manville, New York. A large quantity of Filter Aid is washed by percolation with distilled water that has been

slightly acidulated with hydrochloric acid. This treatment is continued until the effluent is acid to litmus. Washing with distilled water follows until the effluent is essentially neutral, and the Filter Aid is then air-dried. A quantity, usually a quart, is transferred to an air oven at 105°C and kept for use.

PREPARATION OF SAMPLE

Steep water deposits suspended material on standing and the contents should be well shaken before being sampled.

PROCEDURE

Distillation flasks containing 15–20 g of Filter Aid and a test tube are dried to constancy (24 hours) *in vacuo* at 100°C. The flasks on removal from the oven are immediately stoppered (standard taper 40/12) and allowed to cool over P_2O_5. A thin strip of paper is inserted between the stopper and flask to prevent the freezing of the stopper. The flask is then weighed, using a tare weight, and 8 to 12 g of steep water, depending on its Baumé, is quickly transferred by means of a pipette with an open end to the flask, which is then re-weighed. The steep water is rapidly incorporated into the Filter Aid by inserting a glass rod into the test tube and mixing the contents. Seventy-five milliliters of benzene is then added and the mixture is given a further rapid mixing. The flask is then attached to the trap and condenser, the trap is filled with solvent, and distillation is conducted in the manner described for corn. The water is usually completely removed within 24 hours. A correction factor of 0.02 ml is added to the obtained water.

SUGGESTIONS TO AUTHORS

General Arrangement of Manuscript: Send one copy, double-spaced throughout except for footnotes, with about inch-width margins. Place each table on a separate sheet. Throughout the text insert footnotes, single spaced, with a solid rule above and below, just below the lines in which the reference numbers occur. Indicate appropriate places for figures and tables by typing "(Table I)" or "(Table II)" etc., or "(Fig. 1)" or "(Fig. 2)" etc., in the center of the page. Submit the illustrations as separate items with numbers and authors' names on the back, reduced to convenient size. Make a separate list of legends for illustrations.

Tables: For tabulations use the type of arrangement found in previous numbers of CEREAL CHEMISTRY. Suggestions that may be useful in simplifying and reducing the size of tables are as follows: Omit columns if the data represent simple calculations from data in other columns. Omit columns that contain only a few data. Omit data that are of value only to the author. Omit columns that do not show significant variations. Limit tables to approximately 8 columns so that they can be placed horizontally on the page. If the tabulation is small and no correlation is shown, use a "leader" table, one without number and title.

Line Drawings and Photographs: These should have serial figure numbers and legends. Submit copy on 8½ × 11-inch paper or smaller. Large graphs may be reduced by the photostatic or photographic process.

The author should if possible have all line drawings made by a competent draftsman. Use the horizontal scale for independent and the vertical scale for dependent variables. It is usually recommended that omissions in the scales be shown by breaking. Symbols such as circles and triangles cannot be set in type and consequently must be explained in a legend on the drawing. The lettering should be large enough so that after reduction it will be from 1/16 to 1/8 inch high. Use a fine pen for coordinates and a heavier pen for the curves and borders. Avoid waste space; make the graph neat and well balanced. Enclose all sides with border lines.

Glossy photographic prints are best and a width of not less than 5 inches is convenient. The 5-inch width is a standard size and is near to page width. Keep in mind the page width (4¼ inches) in arranging objects to be photographed. Use the vertical page dimension (7 inches) only in cases of necessity.

Text: The title should be specific and long enough to name the factors involved in the investigation. Avoid unnecessary words.

The introduction and review of literature should deal only with those matters *most closely* related to the investigation. To avoid an extremely long review of literature the following methods of elimination are suggested: (1) Cite a competent, recent review and let it serve in place of a similar review. (2) Consider the literature reviewed in the order of relationship to the problem at hand and eliminate the less closely related references.

Methods of statistical analysis do not require explanation. Treat them as you would other standard procedures; that is, interpret the results but do not present the method.

Edit sentences with the object of reducing the number of words. In sentence construction select strong specific subjects, use active verbs as much as possible, and delete all unnecessary modifiers. The language of technical papers is commonly a subject for severe criticism.

Use center headings for the larger sections and divide long sections by means of sideheads. Use only these two types of subdivisions.

Always include a summary, consisting of brief unnumbered paragraphs. Place the acknowledgments between the summary and the list of literature cited. Follow the style of previous issues in citing literature and preparing the list of literature cited. Acknowledge the use of unpublished matter in a footnote or in the text.

Abbreviations: For publications use the abbreviations found in the list of periodicals abstracted in CHEMICAL ABSTRACTS (1936). Do not use periods following abbreviations. Use *ml* instead of *cc*, except for loaf volumes. Abbreviate freely in tables, and if the abbreviations are not standard show their meaning in a footnote.

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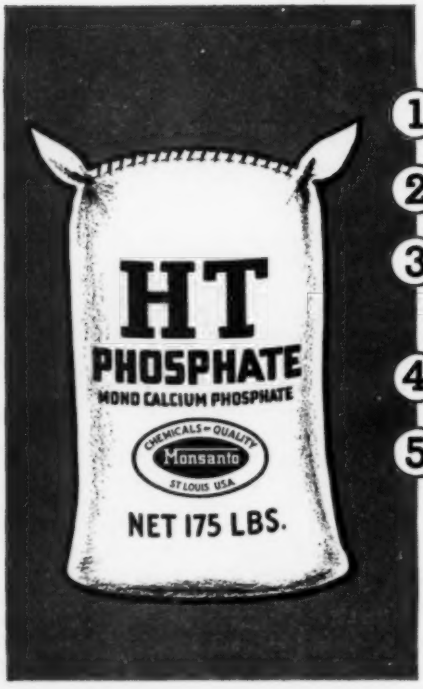

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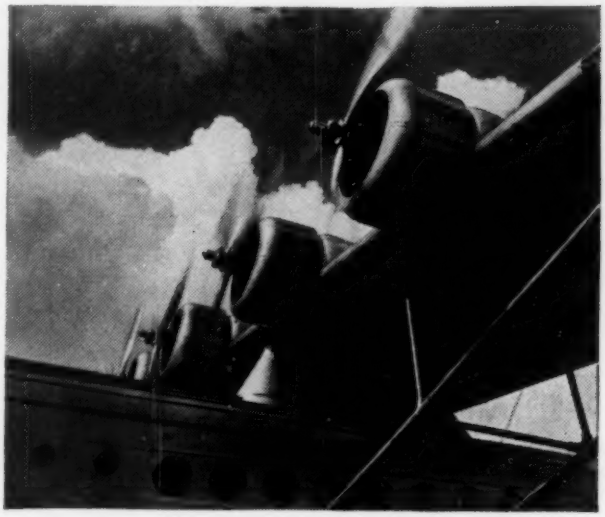

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